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A Management Oriented Model of an Abalone Fishery Whose Substocks are Subject to Pulse Fishing

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Sluczanowski, P. R. 1984. A management oriented model of an abalone fishery whose substocks are subject to pulse fishing. Can. J. Fish. Aquat. Sci. 41: 1008–1014.

I present a model of the exploitation of a fishery whose substocks are subject to periodic fishing during which most fish over the legal minimum length l_R are removed. Managers should find the model useful because the period P between successive fishing visits to each substock is an index to exploitation rate, which is usually more easily measured and controlled than the fishing mortality F. Each management choice of l_R and closure period P affects both long-term biomass yield and egg production. Using a portion of the South Australian abalone (*Haliotis* sp.) fishery as a case study, I conclude that increases in either control variable can be used to increase significantly egg production while causing only a marginal decrease in yield. Longer closures also offer potential economic benefits. Depending on alternative hypotheses regarding the recruitment process, controls presently in use may threaten survival of the stock by reducing fertile egg production to critical levels.

L'auteur présente un modèle de l'exploitation d'une pêche dont les sous-stocks sont sujets à une pêche pulsatoire mendant laquelle la plupart des poissons d'une taille supérieure à la longueur légale minimum (l_R) sont capturés. Ce modèle devrait être utile aux gestionnaires car la période P entre les visites successives à chaque sous-stock est un indice du taux d'exploitation, habituellement plus facilement quantifiable et contrôlable que la mortalité par pêche F. Chaque choix gestionnel de l_R et de la période de fermeture P a une incidence sur le rendement à long terme de la biomasse et la production d'œufs. En utilisant comme cas-type une partie de la pêche de l'ormeau, dans le sud de l'Australie, l'auteur conclut que des accroissement de l'une ou l'autre de ces variables peuvent servir à augmenter la production d'œufs de façon significative tout en ne causant qu'une faible baisse du rendement. De plus longues périodes de fermeture offrent aussi des avantages économiques potentiels. En fonction d'autres hypothèses sur le processus de recrutement, les contrôles actuellement utilisés peuvent mettre en danger la survie du stock en réduisant la production d'œufs fertiles à des niveaux critiques.

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A fishery composed of a number of substocks, each of which is subjected to periodic (pulse) fishing of high intensity, can be described by a model that is more useful to managers than those based on fishing mortality F, the widely used index of rate of exploitation. In such cases, the period between successive fishing visits to each substock often can be used to measure the rate of exploitation, a variable that is more readily estimated than F, and is easier to control in practice, principally through closure policies. Goh (1973), Clark et al. (1973), and Sluczanowski (1976) dealt with the optimal pulse fishing of stocks described by Beverton–Holt models whereas Clark (1976, Chap. 8) reviewed a number of related problems from other disciplines such as timber or cattle production. In this paper I present a similar model dealing with pulse fishing, which was developed to assist the management of the South Australian abalone (*Haliotis* sp.) fishery.

Methods used to control the exploitation of common property abalone fisheries include closed seasons, closed areas, rotating fishing grounds, bag limits, size limits, and licence limitation, singly or in combination (Mottet 1978). For example, a number of Japanese abalone fisheries are regulated by licence limitation with periodic closures and quotas (Noda 1980). Isibasi and Kojima (1979) described a model demonstrating that yield per recruit can often be improved by a suitable combination of age at first capture and periodic closures. Management of the British Columbia, New Zealand, and Californian abalone fisheries are described, respectively, by Breen (1980), Sainsbury (1982), and Wyner et al. (1977). These authors suggested that the concerns of management have been related primarily to the improvement of biomass yield and the economic efficiency of exploitation.

Although the difficulties of estimating stock-recruitment relationships have prevented their inclusion in most management oriented models, the concept of "recruitment overfishing" is well recognized in fisheries management (Cushing 1977). In the absence of adequate recruitment models, it is worth considering how management measures aimed at improving biomass yield affect the number of fertile eggs produced by a substock.

The simultaneous consideration of conflicting goals in the management of a fish stock has been treated by a number of studies (e.g. Peterman 1975). The purpose of this study was to develop a model that describes the influences of two control variables, period P between fishing visits to a substock and legal minimum length l_R, on two indices of exploitation over the long term: biomass yield and production of fertile eggs per recruit.

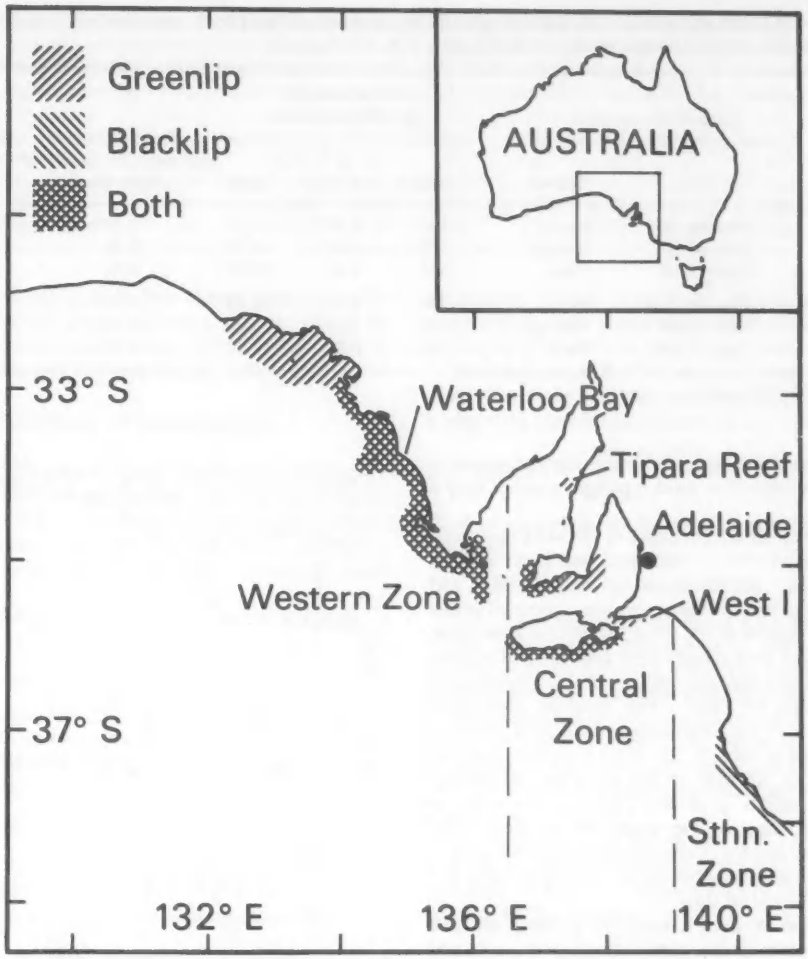

FIG. 1. Distribution of commercial abalone stocks in South Australia.

The objectives also included investigating the sensitivity of the model to parameter uncertainty to identify those biological aspects that require further study.

The South Australian Abalone Fishery

Since the commercial exploitation of abalone began in the mid-1960s, catch rates have remained relatively stable. The main concern of management is to preserve and maintain the stock through a scheme of management also aimed at ensuring the economic viability of the fishery. This goal is sought through policies aimed at influencing the effort expended by divers, the biomass yield per recruit, and continued recruitment. Methods of achieving this include zoning the fishery, limiting the number of divers, and imposing a minimum size limit. In 1981–82, the 35 licenced divers spent 15 300 h diving to collect 1036 t (in shell) of abalone valued at A$3.5 million. The transfer price of a licence was about A$148 000.

Most divers use hookah breathing gear and operate from trailable boats. Their pattern of exploitation around the coast is influenced by distance from home, weather conditions, expected catch rates at various areas, and by the activities of other divers.

Typically, an area containing abalone is fished intensively over a short period until almost all abalone over the legal minimum size limit have been taken; the area is then left to recover. As a result, the period between successive fishing visits to any reef is an index of exploitation rate over the medium term, which is familiar to those associated with the industry on the basis of experience and inspection and can be extracted from available catch and effort statistics. This may vary from 6 mo for highly productive stocks to 3 yr for others, particularly if they are distant from divers' homes.

The greenlip abalone, *Haliotis laevigata* Donovan, and the blacklip abalone, *H. ruber* Leach, comprise the commercially exploited stock in about equal proportions. They are found in shallow water over about 2000 km of coastline (Fig. 1), usually in dense aggregations, often on isolated reefs of various sizes that have been classified into 217 unit areas. Abalone are sedentary and those from one area are thought not to migrate to

TABLE 1. Population dynamics parameters of greenlip *H. laevigata* and blacklip *H. ruber* abalone at three sites in South Australia. Sources of data are described in the text (NA, not available).

Species	Area	Growth	von Bertalanffy growth parameters			Instantaneous mortality rate, M (per year)	Length at first capture, l_R (mm)
			L_∞(mm)	K (per year)	t_0(yr)		
Greenlip	Waterloo Bay	Fast	148	0.59	0.28	0.20[a]	130[a]
	West Island	Average	138	0.48	0.29	0.26	128
	Tipara Reef	Slow	131	0.41	0.03[a]	0.22	128
Blacklip	Waterloo Bay	Fast	144	0.41	−0.07[a]	0.20[a]	NA
	West Island	Average	139	0.34	0.00[a]	0.36[a]	NA
	Tipara Reef	Slow	143	0.32	−0.12[a]	0.21	NA

[a]Unpublished sources.

others. The contribution of recruits from other substocks is probably minimal because they have a pelagic stage of only a few days.

The biology and population dynamics of the stock have been described by Shepherd (1973), Shepherd and Laws (1974), Shepherd et al. (1982), and Shepherd and Hearn (1983), and studies are continuing. Most of the observations reported in this paper on biological aspects of the stock are derived from these papers. Populations of both species have been studied at three sites, chosen to represent the range of growth rates encountered across the waters of the State. The resultant population dynamics characteristics and an indication of the range of values encountered are given in Table 1. The legal minimum limit is a width measurement of 102 mm, so that there may be slightly different minimum length l_R at first capture for abalone at different sites, depending on the shape of the shells. The abalone's life history pattern is characterized by slow growth and a long life, which means that should a catastrophy occur to a substock, recovery would be slow.

Reefs in the Western Zone containing greenlip abalone provide about 37% of the State's total abalone production, and the pattern of their exploitation best satisfies the typical pattern outlined above. The exploitation of these substocks was therefore chosen as the case study with which to demonstrate the model.

The fecundity and reproductive behavior of greenlip abalone have been studied at two sites, West Island and Waterloo Bay. S. A. Shepherd (Department of Fisheries, Box 1625, Adelaide 5001, Australia, unpubl. data) has estimated the fecundity (number of eggs shed annually) E_w of a number of individuals of weight w and length l. The parameters summarizing these data according to equations (2 and 4) presented in the next section are given in Table 2. The length, biomass, and egg production curves for greenlip abalone at Waterloo Bay derived from these parameters are shown in Fig. 2.

Fish stocks in which each female produces a large number of eggs are generally thought to be relatively insensitive to recruitment overfishing, provided the legal age at first capture t_R is above the age at first fecundity t_F. However, the evidence from a number of substocks in South Australia, which have never recovered after intensive fishing although these requirements have been met, casts serious doubts on the validity of this assumption. Reduced recruitment of abalone from stock levels severely reduced by harvesting has also been observed in British Columbia (Breen 1980) and California (Cicin-Sain et al. 1977).

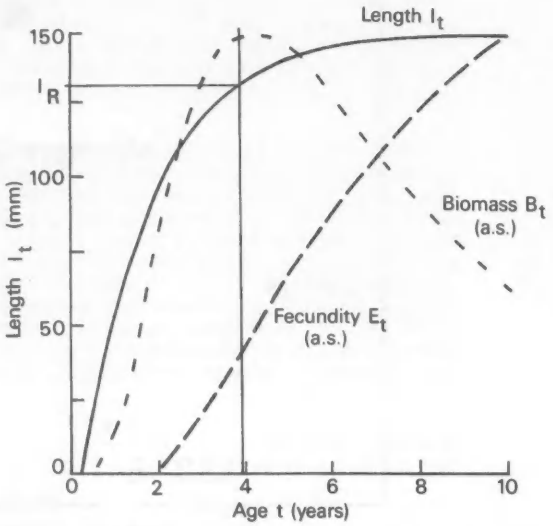

FIG. 2. Length l_t, biomass B_t, and fecundity E_t of greenlip abalone of various ages at Waterloo Bay. (a.s. means "arbitrary scale" chosen to fit all graphs on a single diagram).

As a result of their studies on the Mexican abalone fishery, Doi et al. (1977) suggested that there may be value in maintaining egg production above a certain level.

Thirteen years of data collected at an isolated substock by S. A. Shepherd (unpubl. data) indicate a positive relationship between the biomass of abalone over 4 yr old and subsequent recruitment of 1-yr-olds. Since abalone younger than 4 yr produce relatively few eggs, it follows that future recruitment may be determined by the level of egg production.

Explanations of recruitment failure and its relationship to egg production are mainly speculative at present, but the consequences, should they be valid, are most important and therefore were investigated. The short pelagic phase during which abalone larva must settle suggest that small substocks that exist in areas sparse in suitable substrate may become more vulnerable to recruitment failure if their egg productivity is significantly reduced. Abalone are broadcast fertilizers, which suggests that the more densely the spawners are congregated, the more likely are their eggs to be fertilized. S. A. Shepherd

TABLE 2. Parameters fitted by regression to greenlip abalone data (S. A. Shepherd, unpubl. data) at Waterloo Bay and West Island. Parameters a and b relate weight w_t (gm) to length l_t (mm) (eq. 2), while the fecundity parameters determine fecundity E_t (millions of eggs) (eq. 4).

		Size parameters			Fecundity parameters			
	Reading	$a/10^{-5}$	b	r^2	t_F	$e_1/10^{-2}$	e_2	r^2
Waterloo	30	8.01	3.08	0.97	1.75	0.383	0.279	0.36
West Island	36	1.87	3.39	0.97	1.59	1.511	0.382	0.82

(unpubl. data) has found that abalone exhibit aggregating behavior when they reach about 120 mm (4.53 yr), raising the possibility that abalone smaller than this contribute little to fertile egg production and also subsequent recruitments.

A Model of the Efficiency of Exploitation

I developed a mathematical model similar to the Beverton–Holt model to describe how the biomass yield per recruit and the number of eggs produced by a substock vary with and so depend on the legal minimum length at first capture and the period between intensive fishing visits to the reef, both of which are easily determined.

The relative number of fish in an age class of age t, N_t, is given by the equation

$$(1)\quad N_t = \exp(-Mt) \quad \text{for } 0 \leq t \leq t_\lambda$$

where M is the instantaneous rate of natural mortality per annum and t_λ is the maximum attainable age.

The increase in length l_t of individual abalone was modeled by the von Bertalanffy curve with parameters L_∞, K, and t_0:

$$l_t = L_\infty[1 - \exp\{-K(t - t_0)\}].$$

The weight w_t of individuals was described by the following relationship depending on parameters a and b:

$$(2)\quad w_t = a(l_t)^b.$$

The biomass of an age-class B_t reaches its maximum $B_{\max}$, at critical age t_{mb}, which can be derived by setting the derivative of B_t equal to zero and ensuring that M, b, and K are greater than zero and t_λ is large enough:

$$B_t = N_t w_t$$

$$B_{\max} = \max B_t = B(t_{mb})$$

where

$$t_{mb} = t_0 + \{\ln(bK/M) +\}/K.$$

The control variables considered are the age at recruitment to the fishery t_R (which is manipulated through choice of the legal minimum length l_R) and the period P between successive fishing visits to a reef, which are assumed to deplete it of all abalone above l_R. The number of abalone recruiting to the fishery at length l_R is assumed to be constant and continuous thoughout the year. The assumption can be justified considering the long period over which spawning can take place (about 5 mo of the year) and the effects of differential growth between individuals over the t_R years before they reach legal minimal length l_R. Relaxation of this assumption would overly complicate the model by adding time of year during which fishing occurs as a control variable.

I chose indicators of yield and egg production to be easily comparable (hence proportions of attainable maxima) and independent of the period over which controls operate (hence, division by P to achieve "per annum" units).

The biomass yield efficiency of exploitation $\tilde{B}(t_R,P)$ is the proportion of the attainable maximum $B_{\max}$ that is harvested on a long-term basis by successive fishing visits of period P:

$$(3)\quad \tilde{B}(t_R, P) = \left\{\int_{t_R}^{\min(t_\lambda,\, t_R+P)} B_t dt\right\} \Big/ PB_{\max}.$$

Continuous fishing would provide a continuous yield of $B(t_R)$, and hence,

$$\tilde{B} \to B(t_R)/B_{\max} \quad \text{as } P \to 0.$$

Egg production was modeled in a similar fashion. An early model also assumed that spawning was restricted to several months of the annual cycle. However, this assumption led to an egg production function that was not smooth, since the number of eggs depended on not only the period P but also the time of year when fishing occurred. The following model proved easier to interpret and gave the same results for all but small values of P (<1), where the values were essentially those of the original model, but smoothed.

The fecundity of a fish of age t, E_t, was measured as the average number of fertile eggs produced by an individual of that age during a year. If t_F is the age at first fecundity and e_1 and e_2 are positive parameters, then

$$(4)\quad \begin{aligned} E_t &= 0 && \text{for } 0 \leq t < t_F \\ E_t &= e_1 w_t - e_2 && \text{for } t_F \leq t \leq t_\lambda. \end{aligned}$$

The number of eggs per recruit shed by an age-class during its lifetime up to age t is given by

$$(5)\quad A_t = \int_{t_F}^{t} N_t E_t dt \quad \text{for } t_F \leq t \leq t_\lambda \text{ and}$$

$$A_{\max} = A(t_\lambda)$$

where $A_{\max}$ is the maximum number of eggs per recruit that can be produced by an age-class.

The choice of legal minimum age t_R was restricted by assuming that l_R is chosen such that

$$t_F < t_R < t_\lambda.$$

Therefore, the long-term realized proportion of potential egg production per recruit, $\tilde{E}$, depends on t_R and P according to the equation

$$(6)\quad \tilde{E}(t_R, P) = \left\{\int_{t_R}^{\min(t_R+P,\, t_\lambda)} A_t dt\right\} \Big/ PA_{\max}.$$

The relationships described by the equations above for $\tilde{B}$ and $\tilde{E}$ were translated into a computer program, which generated a table of yield efficiencies and egg production over relevant

TABLE 3. Efficiency of biomass yield and egg production per recruit for greenlip abalone at Waterloo Bay for minimum age at first fecundity $t_F = 1.75$ yr. The maximum biomass of 121.8 occurs at age $t = 4.16$ yr, and the maximum expected eggs per recruit is 2.63 million.

Legal minimum		Biomass yield for fishing period (yr)					Egg production for fishing period (yr)				
Length (mm)	Age (yr)	0	1	2	3	4	0	1	2	3	4
118	2.99	87	95	97	95	92	7	12	18	25	31
122	3.23	92	98	98	95	91	9	15	21	28	34
126	3.51	96	99	98	94	89	12	19	25	32	38
130	3.85	99	99	96	92	86	16	23	30	36	42
134	4.28	100	98	93	88	82	22	29	35	41	47
138	4.85	98	93	88	82	76	30	36	42	48	53
142	5.71	90	84	78	72	66	41	47	52	57	62
146	7.58	67	62	56	52	47	62	66	70	74	77

ranges of t_R and P for any set of input data. This consisted of the von Bertalanffy growth parameters L_∞, K, and t_0, the parameters a and b relating length to weight, the natural mortality M, upper age t_λ, and the fecundity parameters t_F, e_1, and e_2. The integration was carried out numerically and the efficiencies were expressed as percentages.

Results

The stock at Waterloo Bay, analyzed in Table 3, was regarded as representative of those greenlip abalone substocks in the Western Zone that contribute the major portion of its catch. The effects of regulations based on these parameters on other substocks are examined later.

By inspecting Table 3, one can easily assess the situation at a number of reefs, each of which is subject to a common legal minimal length l_R but whose periods between fishing may vary. Consider the present situation of $l_R = 130$ mm. Because the corresponding legal minimum age $t_R = 3.85$ yr is so close to $t_{mb} = 4.16$, the biomass yield is almost 100% for short periods between fishing. Even with 4-yr intervals, 86% of the potential yield is realized. The situation for the long-term production of eggs is less stable. Continuous fishing ($P = 0$) allows only 16% of potential egg production, which can be increased to 42% with 4-yr closures. Suppose that a reef is known to be exploited currently about once annually. The corresponding yield efficiency and egg production are 99 and 23%, respectively. A series of 3-yr closures would reduce the yield by 7% while increasing egg production by 57%. If a significant positive relationship exists between number of eggs and subsequent recruitment to the fishery, then the benefits of increased stocks would soon more than compensate for this reduction.

Similarly, one can examine the effects of possible changes in the legal minimum length l_R. For example, raising l_R to 138 mm would have a major effect on egg production, increasing it by at least 40% at all reefs fished more frequently than once every 2 yr. The long-term reduction in overall catch would be relatively small. Increasing the legal minimum length l_R from 130 mm also could effectively reduce the stock available for exploitation. The asymptotic length of abalone is statistically distributed, and those individuals that would never grow to the larger l_R would also cease to be liable to capture. The model could be extended to account for this.

The biomass yield section of Table 3 contains information analogous to Beverton and Holt's (1957, p. 318) yield isopleth diagram. The response surface highlights the wide ranges of the control parameters l_R and P over which the model is relatively insensitive. For example, the 90% contour line would enclose most of the range of l_R and P presented.

The parameters for greenlip abalone from West Island represent a number of reefs also commercially fished in the Western Zone whose population characteristics are different from those at Waterloo Bay. The efficiency of their exploitation is shown in Table 4 for comparison with Table 3. A legal minimum length of 130 mm is better for this stock as far as egg production is concerned but poorer in terms of meat production. Any increase in l_R will reduce yields in return for a marginal increase in the number of eggs. Most of the abalone would never reach 138 mm and would therefore effectively be excluded from a fishery with such a limit.

Before discussing the implications of these results for management of the Western Zone greenlip abalone stocks, I present an investigation of the sensitivity of the model to key input parameters.

The egg production efficiencies presented in Table 3 were based on the fecundity–weight relationships shown in Fig. 2. The age at first production of eggs likely to influence recruitment t_F was taken as 1.75 yr. The results for two additional choices of t_F are presented in Table 5. The larger one, $t_F = 4.53$, corresponds to a length of about 120 mm of West Island greenlip abalone suggested as a pessimistic but possible alternative (see earlier comments on aggregating behaviour). The effects are most pronounced. If $t_F = 4.53$ yr best describes the actual situation, then at the current legal minimum length $l_R = 130$ mm the egg production for reefs fished more frequently than once every 2 yr is very low (about 1%). The consequences for the survival of the fishery are potentially serious, suggesting that research into these aspects should receive relatively high priority. Raising l_R or imposing closures longer than 2 yr would appear sensible.

The sensitivity of the model to the mortality parameter M was tested by running it for Waterloo Bay greenlip abalone with mortalities higher than its best estimate of 0.20 per annum. The results for biomass yield only are listed in Table 6 because the egg production was almost unaffected. Increased mortalities meant that the peak biomass per recruit B_{max} occurs at an earlier age of individuals t_{mb}. Consequently, the longer one defers fishing after t_{mb}, the more potential yield is lost. This is reflected

TABLE 4. Efficiency of exploitation of greenlip abalone at West Island. The maximum biomass of 65.7 occurs at age 4.20 yr, and the maximum number of eggs produced is 6.15 million.

Legal minimum		Biomass yield for fishing period (yr)					Egg production for fishing period (yr)				
Length (mm)	Age (yr)	0	1	2	3	4	0	1	2	3	4
118	4.31	100	99	94	88	82	24	32	38	45	51
122	4.78	100	96	90	83	77	31	38	45	51	56
126	5.38	95	89	82	76	69	40	46	52	58	62
130	6.22	85	78	71	64	58	51	57	62	66	70
134	7.67	65	58	52	47	42	67	71	75	78	81
136	9.11	47	41	37	33	30	78	82	84	87	89

TABLE 5. Effects on egg production of changes in the minimum age of first fecundity t_F for greenlip abalone at Waterloo Bay.

Legal minimum		Fishing period[a]					Fishing period[b]				
Length (mm)	Age (yr)	0	1	2	3	4	0	1	2	3	4
118	2.99	0	6	13	20	26	0	0	1	6	13
122	3.23	3	9	16	23	29	0	0	2	8	15
126	3.51	6	13	20	27	33	0	0	4	11	19
130	3.85	10	17	25	31	38	0	1	8	15	23
134	4.28	16	23	30	37	43	0	5	13	21	29
138	4.85	25	32	38	44	50	6	14	23	30	37
142	5.71	37	43	49	54	59	21	29	36	43	49
146	7.58	59	64	68	72	75	48	55	60	65	69

[a] t_F/maximum eggs per recruit = 3.00 yr/2.45 million.
[b] t_F/maximum eggs per recruit = 4.53 yr/1.96 million.

TABLE 6. Efficiency of biomass yield for greenlip abalone at Waterloo Bay for varying natural mortalities M (results for $M = 0.20$ are listed in Table 2).

Legal minimum		Fishing period ($M = 0.26$)[a]					Fishing period ($M = 0.38$)[b]				
Length (mm)	Age (yr)	0	1	2	3	4	0	1	2	3	4
118	2.99	93	98	97	93	87	99	98	92	84	75
122	3.23	97	99	97	91	85	100	97	89	80	71
126	3.51	99	99	95	89	82	99	93	85	75	67
130	3.85	100	97	92	85	78	96	88	79	69	61
134	4.28	98	93	87	79	73	90	80	71	62	54
138	4.85	93	86	79	72	65	79	69	60	52	45
142	5.71	81	73	66	60	54	62	53	46	39	34
146	7.58	54	48	43	38	35	33	28	24	20	18

[a] Maximum biomass B_{max} (at age $t_{mb} = 3.76$) = 95.9.
[b] Maximum biomass B_{max} (at age $t_{mb} = 3.22$) = 62.9.

by the rapid decrease in $\bar{B}$ at higher mortalities for increasing values of l_R and P. Research aimed at refining the estimates of M would improve the model's usefulness.

Over the ranges of l_R and P considered, higher values of the maximum attainable age t_λ affect only the denominators of the expressions for $\bar{B}$ and $\bar{E}$ (equations 3 and 6), thus preserving the ratio of any two values in any table used for comparisons. With mortality rate $M = 0.2$, 5% of recruits to the population are alive at age 15, and with infinite t_λ the expected maximum potential egg production per recruit would increase by less than 10%. Therefore, the results are relatively insensitive to a precise choice of t_λ greater than 15 yr.

Discussion

The model demonstrates the consequences on long-term biomass yield and egg production per recruit of combinations of legal minimum length and period between successive fishing visits to an abalone producing area. The greenlip abalone stocks of the Western Zone were chosen as an example because they produce a significant portion of South Australia's catch, because data were available, and also because changes in their management have been suggested by divers' representatives. Similar techniques could be applied to other stocks where the necessary assumptions can be justified.

Management of the stock should take account of the following consequences of my analysis. The present legal minimum length of 130 mm achieves a high yield efficiency for all periods, but leads to an egg production efficiency between 16 and 36%, on the most optimistic assumptions of age at first effective fecundity. Research should aim to establish this age and the validity of a postulated positive egg-production/recruitment relationship. This is of particular importance because if abalone below 120 mm contribute little to subsequent recruitments, as has been suggested, the efficiency of egg production is very low, even down to 1% for reefs fished more than once every 2 yr.

When considering management options, the biological and economic benefits of longer time closures need to be weighed against their disadvantages. Longer closures increase egg production and so reduce the risks of recruitment overfishing. They also offer economic benefits in that longer closures reduce the average annual production only slightly while at the same time significantly reducing costs, since fewer fishing visits are necessary. The disadvantages are enhanced opportunities for poachers and the political and economic consequences of extensive time closure.

Ideally, the population parameters of each substock should be obtained and the overall controls affecting them all should be determined by weighting their effects in relation to their individual productivities. In the absence of a scheme of management in which divers cooperatively designate areas for exploitation at optimal sizes and times, the best solution is probably to divide the zone into a number of areas containing abalone with similar population characteristics and then impose the most appropriate combinations of legal minimum size l_R and period P on each. Although these could be juxtaposed as a form of rolling closures, such management measures may face considerable administrative constraints (Wyner et al. 1977).

The study has shown that the period between fishing visits can be used to provide useful management models for stocks that are periodically harvested down to legal minimum length. A number of sedentary stocks would fit this description. The use of the model is not restricted solely to fisheries where the stocks appear in readily identifiable congregations. It applies equally to any subset of a stock whose pattern of exploitation is one of periodic harvesting down to l_R. If the periods between such harvests are known, then the efficiency of its biomass and egg production can be readily estimated, as well as assessing the effects of possible changes.

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Empirical Relationships between Bacterial Abundance and Chlorophyll Concentration in Fresh and Marine Waters[1]

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Bird, D. F., and J. Kalff. 1984. Empirical relationship between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can. J. Fish. Aquat. Sci. 41: 1015–1023.

A strong, positive empirical relationship was found between bacterial abundance and chlorophyll concentration in fresh and marine waters. Freshwater and marine linear regression equations are statistically indistinguishable. The overall equation is log AODC = 5.867 + 0.776 log chl *a*, r^2 = 0.88, where AODC (acridine orange direct count) is the number of bacteria per millilitre and chl *a* is micrograms of chlorophyll *a* per litre. It is apparent that planktonic bacteria and algae are tightly linked in lakes and the sea. The slope of the regression line, however, shows that bacterial numbers do not increase as rapidly as algal biomass with an increase in nutrient concentration. We suggest that this disproportionately smaller increase in bacterial numbers need not signify a smaller role for bacteria in lake metabolism with increasing nutrient availability, if bacterial productivity per unit bacterial biomass increases as total bacterial biomass increases between systems.

Les auteurs ont trouvé une relation empirique marquée entre l'abondance des bactéries et la concentration de la chlorophylle dans les eaux douces et salées. Les équations de régression linéaire pour les deux milieux sont statistiquement indiscernables ; l'équation générale est log AODC = 5,867 + 0,776 log chl *a*, r^2 = 0,88, où AODC (numération à l'orangé d'acridine) représente le nombre de bactéries par millilitre et chl *a*, les microgrammes de chlorophylle *a* par litre. Il semble que les bactéries et les algues planctoniques sont étroitement liées en milieux lacustre et marin. Toutefois, la partie de la régression linéaire montre que le nombre de bactéries n'augmente pas aussi rapidement que la biomasse algale quand la concentration de bioéléments croît. Les auteurs formulent l'hypothèse que cette croissance moindre du nombre de bactéries ne signifie pas nécessairement que celles-ci jouent un plus petit rôle dans le métabolisme lacustre où existe un apport croissant de bioéléments, si la productivité bactérienne par unité de biomasse bactérienne s'élève en même temps que la biomasse bactérienne totale augmente entre les systèmes.

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Many authors have suggested that bacterial abundance increases with the trophic state of freshwater and marine systems (Azam et al. 1983; Faust and Correll 1976; Ferguson and Palumbo 1979; Fuhrman et al. 1980; Godlewska-Lipowa 1976, 1979; Hobbie and Wright 1979; Jones 1972, 1977; Kuznetsov 1970; Rao et al. 1979; Saunders 1980; Silvey and Roach 1964; Straskrabova 1968). Straskrabova (1968) and Kuznetsov (1970) produced tables linking bacterial direct counts to trophic classification. In a study of a large number of lakes and reservoirs in Poland, Godlewska-Lipowa (1976) showed that ranking lakes by bacterial abundance also ranks them by degree of eutrophication and organic matter pollution. A similar multi-lake study by Spencer (1978) uncovered a weak but significant positive correlation between bacterial numbers and both total nitrogen (R^2 = 0.31) and total phosphorus (R^2 = 0.16) in New Zealand lakes. Aizaki et al. (1981), and more recently Linley et al. (1983), first put the relationship into an explicitly predictive form, in fresh and marine systems, respectively. Our objective was to quantify and test empirically the hypothesized relationship between bacterial abundance and trophic condition, measured as the concentration of chlorophyll *a* in surface waters.

Aizaki et al. (1981) found a strong statistical relationship between bacterial number and chlorophyll *a* in Japanese lakes. We attempted to extend that result in two ways. First, we tested the general applicability of their findings to other lakes and to the sea, using both literature values and observations collected in 11 Quebec lakes spanning a wide trophic range. Second, we considered the mathematical form of the resulting equations in the light of current ideas about the role of bacteria in aquatic systems. The work was undertaken to provide a firm basis for the prediction of bacterial abundance but would also allow us to draw inferences about algal–bacterial interactions.

Methods

Literature Values

Records of simultaneous observations of bacterial abundance and chlorophyll concentration were collected from the literature (Bunch 1979; Chapra and Dobson 1981; Coveney 1982; Daley et al. 1981; Field et al. 1980; Fuhrman et al. 1980; Hodson et al. 1981; Kilham 1981; Kogure et al. 1980; Krempin and Sullivan

[1]Contribution No. 115 of the McGill Limnology Research Centre, Lake Memphremagog Project.

1981; MacIsaac et al. 1980; Meyer-Reil et al. 1979; Rao et al. 1979; Rao et al. 1981; Riemann et al. 1982; Schleyer 1981; Väätänen 1980; Valdes and Albright 1981).[2] We restricted the study to those papers that used the acridine orange– epifluorescence direct count (AODC) method of Francisco et al. (1973) as modified by Hobbie et al. (1977), a method that is now in general use for bacterial enumeration. The AODC method has the needed contrast, not attainable even with the transmitted light–acridine orange technique, to visualize the very numerous small bacteria (0.1–0.6 μm) and to distinguish them from detritus (Daley 1979). Counts using electron microscopy have confirmed that all bacteria trapped on the 0.2-μm filter are counted using AODC (Bowden 1977; Larsson et al. 1978). Loss of the small bacteria that pass through this filter is accepted because these bacteria contribute little to total bacterial biomass, and because the low flow rate of finer filters makes them unsuitable for routine use (Zimmermann 1977).

Bacterial observations taken at a depth of 1 m were used when possible. This was the most common depth for bacterial sampling, making up 62% of the data used, and selection of these samples created a more homogeneous data set. However, since the use of 1-m samples exclusively would have reduced the data to only 23 points, we accepted AODC data collected elsewhere in the euphotic zone together with chlorophyll *a* measurements there. Some data represent single samples of both bacteria and chlorophyll at discrete depths (39%), some represent the summer average at a discrete depth (21%), some represent the summer average of epilimnetic integrated samples (39%), and one is an annual average. Single-sample observations are more likely to be in error than many-sample averages; we looked for the effect of this error on our analysis by using a weighting method, described under Results. We accepted the results of any spectrophotometric or fluorometric analyses of chlorophyll *a* concentration. The specific routine used for chlorophyll measurement was outlined for 54% of the literature observations. Of these, 15% of the marine and 50% of the freshwater estimates were corrected for phaeopigment interference. However, we could find no systematic differences between corrected and uncorrected observations in any subsequent analyses.

We performed a simple linear regression analysis on the data using both SAS (Helwig and Council 1979) and BMDP9R (Dixon 1981), depending on the statistical features desired. The data were transformed to logarithms to more closely satisfy the assumptions of ordinary least squares (Draper and Smith 1981). Regression coefficients were tested for significance following Edwards (1967); confidence limits for coefficients were calculated according to Draper and Smith (1981). An independent bacteria–chlorophyll data set (27 points) collected from the literature following the original analysis was used to test the model's predictive capabilities. Nonparametric testing of these results followed Conover (1971). Marine observations from this same data set were used to test the predictive model of Linley et al. (1983).

When appropriate, we compensated for two possible types of error in using regression estimates. First, the simple antilog of the value predicted by a log-transformed regression equation underestimates the expected value of the untransformed variable (Land 1972). To correct for this bias, the antilog of the prediction must be multiplied by a correction factor calculated as antilog (1.1513 × RMS), where RMS is the residual mean square of the regression (Neyman and Scott 1960). We calculated correction factors for all predictive equations presented. Unbiased confidence limits for the mean and for individual predictions were calculated using Cox's direct method (Land 1972). Second, when there is error in the independent variable, the ordinary least squares slope estimate can underestimate the slope that would be obtained if error were absent. In the usual case in ecology where the magnitude of independent variable error is unknown, there is no certain method of determining this underlying ideal or "functional" slope (Sprent and Dolby 1980). We derived the model II, geometric mean slopes recommended by Ricker (1973) in an attempt to compensate for this bias when we were seeking functional relationships.

[2]Complete data sets used in the analyses and their sources are available, at a nominal cost, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada K1A 0S2.

Quebec Lakes

Eleven Quebec lakes were sampled for bacteria during July–August 1982 at 1 m with a Van Dorn bottle. The bacteria were preserved in buffered formalin and counted according to Hobbie et al. (1977). We stained 2-mL samples with 0.2 mL of 0.1% acridine orange and filtered the samples through dyed (Irgalan black) 0.2-μm pore size Nuclepore filters. The filters were mounted in oil and examined at 1000× with a Leitz Orthoplan microscope equipped with Ploem Pack 2,an HBO 100-W lamp, Cube G, 3-mm excitation filter BG 12, mirror 510, and barrier filter 515. We counted 10–50 randomly selected fields from all parts of the filter so that confidence limits for the mean count were always less than 10% of the sample mean.

Chlorophyll values for the lakes (Pace 1984) are the arithmetic means of monthly samples taken throughout the summer, analyzed according to Strickland and Parsons (1972), and corrected for phaeopigment content with the equations of Lorenzen (1967). Samples were taken with a tube at one station over the deepest part of the lake. Depending on the depth of the lake, samples were integrated over depths of between 0 and 8 and 0 and 10 m. Because of the importance of phosphorus in determining different aspects of lake productivity, total phosphorus samples were collected on the same dates and at the same depths as the chlorophyll samples and analyzed according to Johnson (1971).

Results

We found a highly significant relationship between AODC data from both the freshwater and marine literatures and the associated chlorophyll *a* concentrations (*F*-test: $P < 0.0001$) (Fig. 1) (Table 1). The overall relationship using data from both sources is

$$(1) \quad \log \text{AODC} = 5.855 + 0.844 \log \text{chl } a$$

where AODC is acridine orange direct count (number of bacteria per millilitre) and chl *a* is chlorophyll *a* concentrations (micrograms per litre). There was a weak but significant negative correlation between the absolute value of the residual error term associated with a particular point and the number of separate observations represented by the point (*t*-test: $P < 0.05$). We reanalyzed the data, weighting each data point by the number of separate bacteria– chlorophyll samples it represen-

ted. This procedure reduced the effect of single sample observations on the coefficient estimates and emphasized multisample observations. The weighted least squares analysis lowered the slope:

(2) $\log \text{AODC} = 5.877 + 0.783 \log \text{chl } a.$

The major reason for the change in the equation was revealed by scrutiny of the individual residuals using Cook's distance measure, D_i (Cook and Weisberg 1982). The numerical value of Cook's distance associated with a particular observation is a simple and objective index of the influence of that observation on the overall regression estimates (Cook 1977). The value of D_i associated with an individual observation reflects the amount of change that would occur in the regression equation coefficients were that observation to be removed from the analysis. A high D_i associated with Lake Elmenteita, Kenya (AODC: Kilham 1981; chlorophyll: Kalff 1983) showed that the point was having a strong influence on the equation. Cook's D_i for Elmenteita was 1.22; a value greater than 1.00 in this case indicates a highly influential point (Cook and Weisberg 1982, p. 118). We recalculated the coefficients excluding this point; the revised equation is

(3) $\log \text{AODC} = 5.867 + 0.776 \log \text{chl } a.$

This corresponds very closely to the weighted least squares regression and is probably a more accurate representation of the relationship between bacterial abundance and chlorophyll concentration than the original equation using all data (equation 1). By excluding Lake Elmenteita from the analysis, its influence was removed, although the useful range of the equation was reduced at the same time. The range of chlorophyll *a* concentrations over which this equation (equation 3) is now useful is from 0.05 to 120 μg/L.

We split the data into components for hypothesis testing. First, the slope of the equation using just freshwater data is not significantly different from the one obtained by Aizaki et al. (1981) using data from Japanese lakes (Fig. 1; Table 1) although the probability was borderline (*t*-test: $0.05 < P < 0.1$). However, the intercept difference is highly significant (*t*-test: $P < 0.0001$). Next, the marine data (Fig. 1) were analyzed separately (Table 1). Neither the slope nor intercept was significantly different from the freshwater literature model (intercept (*t*-test): $0.2 < P < 0.4$; slope (*t*-test): $0.5 < P < 0.6$), whereas again the intercept was significantly higher than the Aizaki model (intercept (*t*-test): $P < 0.0001$; slope (*t*-test): $0.3 < P < 0.4$). When we tested the freshwater literature equation against the Quebec lakes equation (Tables 1 and 2; Fig. 2), we found that again the slopes were not statistically distinguishable (*t*-test; $0.2 < P < 0.4$) but that the intercepts were different (*t*-test: $P < 0.001$).

We used a dummy intercept variable to get an estimate of the average difference between observations from the literature and from Aizaki et al. (1981) (Draper and Smith 1981). This variable has a value of 1 for all Aizaki data points and 0 for the others that serve as reference points. When used in a multiple regression, a dummy variable has the effect of moving the center of mass of the group of observations to lie on the reference regression line. The coefficient of the dummy is then the average difference in the dependent variable values between groups. The resulting equation is

(4) $\log \text{AODC} = 5.953 + 0.692 \log \text{chl } a - 0.554 D_1$

where chl *a* is chlorophyll *a* concentration (micrograms per litre), AODC is number of bacteria per millilitre, and D_1 is the dummy variable. The dummy variable coefficient reveals that on average the Aizaki et al. (1981) bacterial counts are about 60% lower than those from the literature at comparable chlorophyll concentrations. The difference seems to stem from differences in methodology (see Discussion).

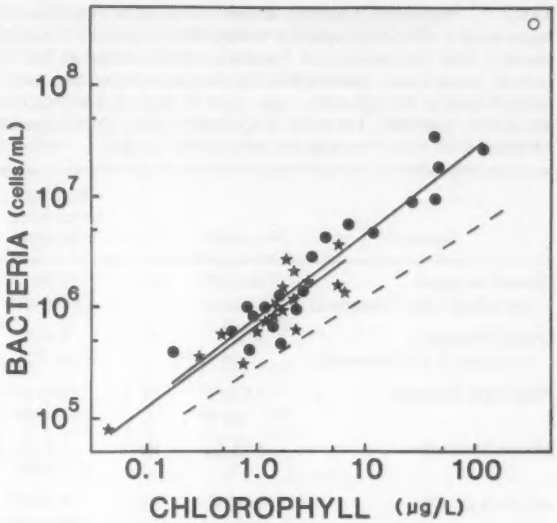

FIG. 1. Scatter diagram of the points obtained from the literature used to derive regressions of AODC of bacterial abundance and chlorophyll *a* concentration. Solid lines represent separate freshwater and marine regressions we derived; the broken line is from Aizaki et al. (1981). Closed circles represent freshwater data; open circle represents Lake Elmenteita, Kenya (Kilham 1981; Kalff 1983); stars represent marine data. Equations are given in Table 1.

The statistical relationships between bacteria and total phosphorus concentration, and between chlorophyll and total phosphorus concentration (Table 1), were determined using the Quebec data (Table 2) in order to approach algal–bacterial interactions from a different perspective. Bacteria–phosphorus relations have become of interest recently following indications that bacteria might play an unexpectedly large, even dominant, role in orthophosphate uptake in situ (Faust and Correll 1976; Harrison et al. 1977; Krempin et al. 1981; Lean and White 1983). The slope of the bacteria–phosphorus regression was significantly less than 1 (*t*-test: $P < 0.01$), while the slope of the chlorophyll–phosphorus regression was not different from 1. This means that though the algal biomass rises at the same rate as total phosphorus concentration, the bacteria make up a smaller proportion of the total phosphorus standing stock at eutrophic than at oligotrophic sites.

Bacterial and algal data collected from the literature since the original analysis was done were used to test the model (equation 3). Two observations out of 27 fell outside the prediction confidence intervals (Table 3; Fig. 3), which is not an unexpected number (χ^2-test: $P > 0.4$).

Particular results are of some interest. First, the bacterial concentrations observed at elevated chlorophyll levels in estuarine waters (Hoppe 1983) were below the predicted values 9 times out of 10. It is likely that the sampling period for these observations (winter–spring bloom) is responsible for this discrepancy because chlorophyll levels were then elevated above their mean levels. However, we have no long-term

TABLE 1. Regression equations derived in this paper, with descriptive statistics. Variables are AODC (acridine orange direct count, no. of bacteria/mL), chl *a* (chlorophyll *a* concentration, μg/L), BB (bacterial biomass, μg C/L), and TP (total phosphorus concentration, μg/L). All variables were log-transformed. Confidence limits around an individual prediction can be calculated using mean X, ΣX^2, information on the residual mean square contained in the correction factor (see text), and standard confidence limits formulae. Predictions of the equations, retransformed to the arithmetic scale, must be multiplied by the correction factor given in order to correct for a bias inherent in the use of log-transformed equations. The model II (geometric mean) slope is provided as an estimate of the functional or "true" numerical relationship between variables when there is error in the independent variable X. ***$P < 0.001$; ****$P < 0.0001$.

Source	*Y*, *X*	*N*	Intercept (confidence limits)	Slope (confidence limits)	r^2	Mean X	ΣX^2	Correction factor	Model II slope
Overall literature (including Lake Elmenteita)	AODC, chl *a*	40	5.85 (±0.080)	0.844 (±0.093)	0.90****	0.430	29.5755	1.132	0.89
Overall literature (excluding Lake Elmenteita)	AODC, chl *a*	39	5.867 (±0.072)	0.776 (±0.094)	0.88****	0.375	22.998	1.105	0.83
Freshwater literature	AODC, chl *a*	20	5.911 (±0.120)	0.763 (±0.125)	0.90****	0.591	18.393	1.114	0.80
Marine literature	AODC, chl *a*	19	5.835 (±0.096)	0.736 (±0.194)	0.79****	0.148	4.6047	1.099	0.83
Aizaki et al. 1981	AODC, chl *a*	23	5.445 (±0.094)	0.630 (±0.088)	0.92****	0.748	26.074	1.066	0.66
Quebec lakes (this study)	AODC, chl *a*	13	6.277 (±0.190)	0.569 (±0.270)	0.66***	0.611	6.6624	1.087	0.70
Es and Meyer-Reil 1982	BB, AODC	10	−3.80 (±1.77)	0.79 (±0.30)	0.82***	5.878	356.3	1.599	0.87
Quebec lakes	AODC, TP	12	5.953 (±0.229)	0.663 (±0.206)	0.83****	1.052	14.782	1.036	0.73
Quebec lakes	chl *a*, TP	12	−0.486 (±0.272)	1.043 (±0.245)	0.90****	1.052	14.782	1.049	1.098

TABLE 2. Bacterial abundance (millions of cells/mL), chlorophyll concentration (μg/L), and total phosphorus (TP) concentration (μg/L) in 11 Quebec lakes. These values were used in the Quebec Lakes regressions of Table 1.

Lake	AODC	chl *a*[a]	TP[a]
Waterloo	13.4	35.0	59.7
Brome	6.50	4.0	14.6
Argent	4.47	2.2	10.7
Brompton	3.56	2.0	6.0
Bowker	1.10	1.3	3.2
Orford	3.00	1.5	4.8
Massawippi	5.72	4.2	12.7
Magog	7.75	13.6	47.4
Lovering	2.85	2.5	7.0
Maryjo	2.08	4.0	—
Memphremagog			
North Basin	3.80	5.0	9.1
Quinn Bay	4.90	4.6	10.0
South Basin	5.86	5.6	12.8

[a]Pace 1984, arithmetic means.

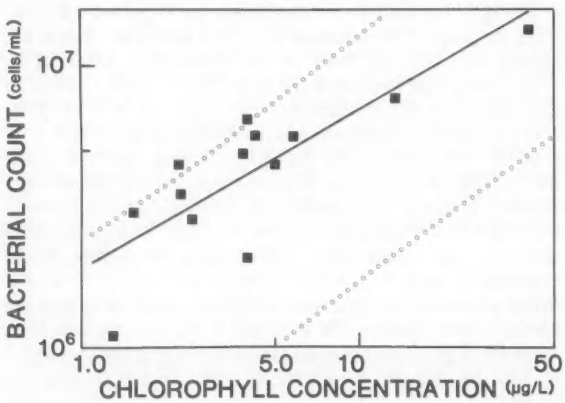

FIG. 2. Bacterial count (AODC) and chlorophyll concentration in 11 Quebec lakes. Solid line is the line of best fit to these data; dotted lines are 95% confidence limits for predictions of the freshwater literature equation (Fig. 1).

bacteria–chlorophyll observations at productive marine sites to corroborate this and therefore cannot be sure that the model does not overestimate bacterial abundance in such cases. Second, the overestimate of bacterioplankton numbers in eutrophic Lake Norrviken (Bell et al. 1983) can be considered a failure of the model in this case (*t*-test: $P < 0.001$). Bell et al. (1983) noted the low number of bacteria in the lake and suggested that an allelopathic interaction with blue-green algae in late summer might be responsible. Other blue-green dominated lakes are not similarly affected (Coveney 1982; Hamilton-Galat and Galat 1983; Kilham 1981; Riemann et al. 1982) so that special circumstances must be involved. The model performed well for the other observations in Table 3. Observations were evenly distributed above and below the prediction line (binomial test: $P > 0.20$) and there was no apparent bias at low or high bacterial

TABLE 3. Observed total bacterial abundance at marine, estuarine, and inland sites from the published literature, with predicted abundance from equation 3 and 95% confidence limits. Abundances are given as millions of cells/mL; *N* refers to the total number of separate observations pooled to get an average value.

Location	Observed bacterial abundance	Predicted abundance	Lower and upper 95% confidence limits	*N*	Source
Marine and estuarine					
Kiel fjord					
Haten	2.60	4.16	1.49–9.48	1	Hoppe 1983
Friederichsort	2.48	4.44	1.59–10.1	1	Hoppe 1983
Laboe	1.11	4.09	1.47–9.31	1	Hoppe 1983
Feuerschiff	1.05	1.66	0.60–3.75	1	Hoppe 1983
Kieler Bucht Mitte	1.03	1.60	0.58–3.63	1	Hoppe 1983
Schlei fjord					
Gr. Breite	14.09	31.0	10.5–75.1	1	Hoppe 1983
Missunde	9.81	23.6	8.08–56.5	1	Hoppe 1983
Kappeln	9.11	17.8	6.16–42.1	1	Hoppe 1983
Schleimünde	2.46	1.34	0.48–3.03	1	Hoppe 1983
Boknis Eck	1.78	2.43	0.88–5.51	1	Hoppe 1983
Tvarminne archipelago					
Storfjärd	2.0[a]	3.29[a]	1.31–8.29	14	Väätänen 1982
Maskskär	1.7[a]	1.86[a]	0.74–4.65	14	Väätänen 1982
Cape Hatt, N.W.T.	0.386	0.369	0.13–0.85	108	Bunch et al. 1981
Kiel Fjord	3.09	4.80	1.72–10.9	13	Bölter et al. 1977
Kiel Bight	1.50	1.32	0.48–2.99	12	Bölter et al. 1977
St. Croix, Virgin Islands	0.745	1.05	0.38–2.39	10	Lasker et al. 1983
Freshwater					
Merrill Lake	0.5	0.32	0.11–0.74	1	Wissmar et al. 1982
McBride Lake	0.6	1.00	0.36–2.25	1	Wissmar et al. 1982
June Lake	0.1	0.14	0.05–0.32	1	Wissmar et al. 1982
Blue Lake	0.8	0.55	0.20–1.25	1	Wissmar et al. 1982
Lake Norrviken	1.55	8.54	3.02–19.8	4	Bell et al. 1983
Pyramid Lake	2.93	2.05	0.74–4.65	17	Galat et al. 1981; Hamilton-Galat and Galat 1983
Lake Gardsjön	1.31	0.84	0.30–1.92	16	Olsson 1983; Johansson 1983
Lake Vechten	1.89	4.78	1.71–10.9	16	Blaauboer et al. 1982
Eastern Lake Erie	2.00	1.82	0.72–4.49	6	Lean et al. 1983
Central Lake Erie	3.93	2.73	1.07–6.76	6	Lean et al. 1983
Lake Tystrup	4.10	10.50	3.69–24.49	17	Riemann 1983

[a]Geometric means.

concentrations (2 × 2 contingency table: $P > 0.60$). The mean ratio of predicted abundance to observed abundance was not significantly different from 1.0 for either freshwater (ratio = 0.96; *t*-test; $P > 0.50$) or marine data (ratio = 1.17; *t*-test: $P > 0.20$).

We used marine observations from the independent data set (Table 3) to test the predictions of a model developed by Linley et al. (1983). Their equation predicts AODC from chlorophyll *a* concentration, based on data the authors collected at seven upwelling and coastal marine stations. The 13 observations from the independent set that fell within the range of their equation (0.10–26.22 μg chl *a*/L) all had more bacteria than predicted by their equation (Fig. 3), and 11 of the 13 were outside that model's 95% prediction confidence limits (χ^2-test: $P = 0.0$).

Discussion

Bacteria and phytoplankton are apparently quantitatively tightly linked both in lakes and the sea. Nevertheless, some considerable scatter remains to be explained and the confidence limits for an individual prediction are wide (Fig. 3). Reasons for this scatter probably include temporal changes in the relationship between chlorophyll *a* and algal biomass (Granberg and Harjula 1982), differences in bacterial cell volume, within-lake plankton dynamics, variation in allochthonous carbon loading, and systematic errors resulting both from differences in microscope configuration (Daley 1979) and from between-operator variability. The systematically lower counts by Aizaki et al. (1981) are at least partially attributable to their use of 0.4-μm, undyed Nuclepore filters (M. Aizaki, pers. comm.) rather than the 0.2-μm, dyed filters recommended (Hobbie et al. 1977) and used by all others in this data set. Cole et al. (1982) noted that one quarter to one half of the bacteria trapped on a 0.2-μm filter passed through a 0.4-μm filter. It appears that on average, three times more bacteria would have been counted by Aizaki et al. (1981) had they used the finer filters. The Japanese lakes were excluded from our overall equation, since any systematic changes in bacterial diameter along a trophic gradient, allowing greater or lesser proportions of bacteria through the larger pores, would bias the slope estimate. Why our own counts should be significantly higher than literature values is unclear, except that we were exceedingly concerned not to miss even the smallest

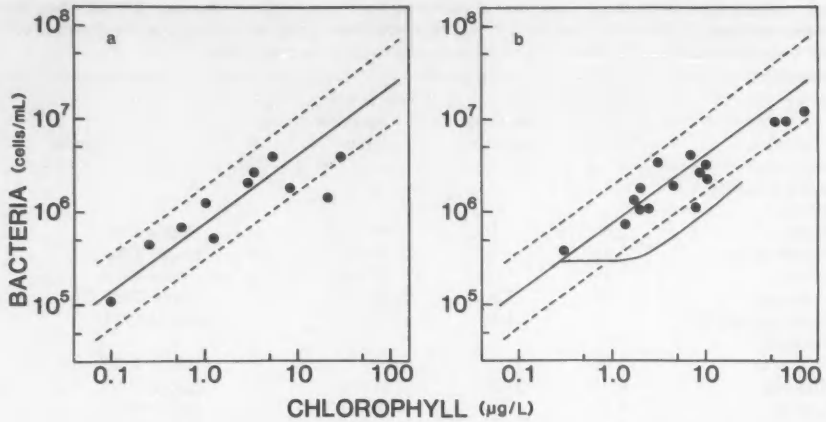

FIG. 3. Comparison of literature regression line (equation 3) with observations collected from the literature (Table 3) subsequent to the original analysis. Broken lines are 95% confidence limits for an individual prediction. (a) Observations from inland waters; (b) observations from marine and estuarine waters. The lower curved line in Fig. 3b corresponds to the predictive equation of Linley et al. (1983).

bacteria that are visible only as minute blurs of light, and we may have counted too many questionable particles in our zeal. Alternatively, our midsummer bacterial observations may overestimate growing season means. The lower and higher intercepts of the Japanese and the Quebec lakes, respectively, are probably due to differences in the bacterial count and not to differences in chlorophyll data, since regressions of chlorophyll on total phosphorus for both cases were indistinguishable from each other and from established relations (Schindler 1978; Vollenweider and Kerekes 1980).

Lake Elmenteita could not be justifiably removed from the data set on the basis of prior information. However, its remoteness from the other points in the sample space accorded it considerable influence in the analysis. Elmenteita's chlorophyll level on the date sampled, measured within 3 wk of the bacterial sampling, was 367 µg/L, while the next highest level in our data set was another Kenyan lake at 120 µg/L. We decided that until adequate coverage is obtained of lakes with chlorophyll concentration greater than 120 µg/L, estimation and prediction beyond that point is premature.

The slope of the overall regression equation, at about 0.78 (model I) or 0.83 (model II), means that the increase in the number of bacteria per unit volume does not keep pace with the increase in chlorophyll *a* as lake trophy changes. This is not peculiar to the bacteria but has also been observed for the crustacean zooplankton (McCauley and Kalff 1981) and for fish (reanalysis of equations in Hanson and Leggett (1981), and Jones and Hoyer (1982) on volumetric yield basis). If bacteria are dependent on primary production for reduced carbon, then there is no obvious reason why this disproportionately lower increase should occur. It was suggested by Pedros-Alio and Brock (1982) that bacterial cell size is positively correlated with trophy, so that bacterial biomass would increase more rapidly than number. Although this would help to explain the discrepancy, we found no evidence for such a relationship (Fig. 4). Indeed, based on the scanning electron microscope cell-size estimates there seems to be a trend toward smaller bacteria in eutrophic waters (*t*-test on slope: $P < 0.05$) although its statistical significance depends on the cluster of Plon lakes (Krambeck et al. 1981) in the lower right corner of Fig. 4 and should probably be confirmed at other eutrophic sites before being accepted. Care was taken during this analysis to distinguish biomass estimates based on epifluorescence microscopy from those based on scanning electron microscopy, which have been shown to be significantly different using identical bacterial samples (Fuhrman 1981). There was no significant relationship between cell size and abundance when only epifluorescence estimates were used. Furthermore, an analysis of data collected from the marine literature by Es and Meyer-Reil (1982) showed the relationship between biomass and abundance to be indistinguishable from linear over a 1000-fold range in bacterial abundance (Table 1). Therefore it is unlikely that the use of abundance rather than biomass as dependent variable has biased the conclusion that chlorophyll concentration and bacterial standing stock do not increase in parallel along a trophic gradient.

Alternatively, it is possible that bacterial abundance is not a proportionate measure of biological activity. It may be that bacterial productivity per unit bacterial biomass increases at a faster rate with increasing trophy than primary productivity per unit chlorophyll. This would mean that bacterial productivity would be approximately linearly related to primary productivity, in interlake comparisons, and that the decline in bacterial numbers relative to chlorophyll concentration is a necessary consequence. Unfortunately, there is still considerable uncertainty in measurements of bacterial productivity. There are at least nine methods in current use (Newell and Christian 1981) and some of these give widely different results (Cole et al. 1982). It is, therefore, not yet possible to test this hypothesis. However, it appears that the relationship between daily primary productivity and chlorophyll *a* concentration is roughly linear between lakes (Smith 1979). If bacterial productivity is linearly related to algal productivity, it must follow that bacterial productivity increases disproportionately faster than bacterial density, given our algal–bacterial equations. This might occur if bacteria were more heavily grazed in eutrophic than in oligotrophic systems (cf. Gliwicz 1969; Beaver and Crisman 1982) or subject to higher sedimentation losses (Pedros-Alio

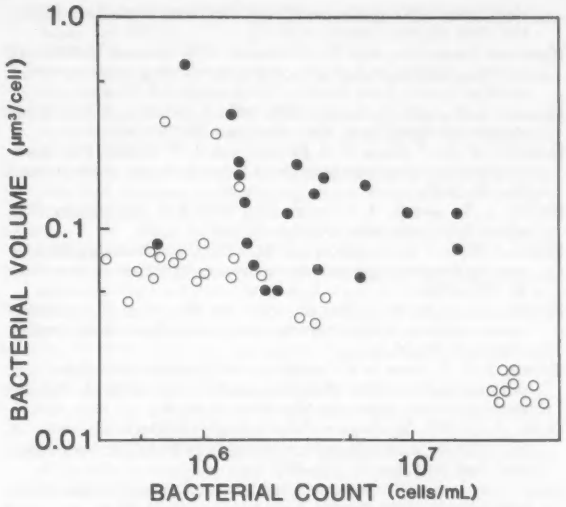

FIG. 4. Relationship between bacterial cell volume and bacterial abundance. Solid circles represent observations made using acridine orange–epifluorescence; open circles represent observations made using scanning electron microscopy.

and Brock 1982; Cole et al. 1982), or if fewer bacteria were dormant and, if so, for shorter periods in eutrophic systems (cf. Morita 1982).

The form of the empirical relationship between bacterial abundance and total phosphorus concentration in Quebec lakes (Table 1) is of considerable interest because bacterial orthophosphate uptake has recently been shown to be important to the cycling of phosphorus in lakes (Currie and Kalff 1984; Lean and White 1983), estuaries (Faust and Correll 1976), and marine coastal waters (Harrison et al. 1977; Krempin et al. 1981). It is known experimentally (Faust and Correll 1976; Mayfield and Inniss 1978; Rhee 1972) and might have been predicted from body size considerations (Smith and Kalff 1982) that bacteria have a decided advantage over the algae in orthophosphate uptake. Yet the epilimnetic bacteria of eutrophic waters appear to contain a smaller proportion of the total phosphorus (TP) present in the system than do the bacteria of oligotrophic waters. This follows from the curvilinearity of the relationship between AODC and total phosphorus for the Quebec lakes (Table 1: model I slope = 0.66; model II slope = 0.73). The slope of the regression line linking chlorophyll concentration to total phosphorus is greater than that linking AODC to total phosphorus, suggesting that as total phosphorus increases, an increasing proportion moves into the algae relative to that of the bacteria. If the bacteria are not subject to disproportionately higher loss rates and if their specific production does not increase with trophic state, then they must be limited by some factor that does not increase at the same rate as phosphorus availability with trophy.

A regression equation linking bacterial counts to chlorophyll concentration in several marine locations has recently been published by Linley et al. (1983). Their model, based on data from the English Channel and an upwelling region east of South Africa, yields predictions of bacterial abundance that are on average 3.0 times lower than observed abundances at the independent sites compatible with our model (Fig. 3). The Linley et al. (1983) model and our literature model are both internally consistent yet they yield mutually incompatible predictions. If the differences are not methodological, they point to (more interesting) biological differences between the sites sampled by Linley et al. (1983) and the sites described by our literature-based equation. In this case the distinguishing attributes are not known.

Lakes can be grouped into trophic categories according to bacterial abundance using a chlorophyll *a* based classification (Forsberg and Ryding 1980) and the regression equation (Table 1). Oligotrophic waters should contain less than 1.7 million bacteria/mL, mesotrophic waters 1.7–6.5 million/mL, and eutrophic waters more than 6.5 million/mL. A comparison of these ranges with literature values based on older direct count methods is difficult because the trophic classification criteria used there were often not defined (e.g. Kuznetsov 1970, p. 122; Sorokin 1972). However, by converting Romanenko's (1973) Latvian lakes primary production rates to chlorophyll *a* concentrations (Smith 1979) an approximate comparison can be made. This reveals that acridine orange–epifluorescence direct counts are 2–7 times higher than Romanenko's transmitted light counts in oligotrophic and mesotrophic lakes and up to 20 times higher in eutrophic lakes. Unfortunately, there does not seem to be any simple conversion method that would make the older bacterial values comparable.

The purpose of the study was to demonstrate that aquatic bacterial abundance is predictable, albeit with some uncertainty for the present. It was intriguing to discover that bacteria relate to algal biomass in much the same way in the sea and in freshwater. The surprising fact that bacterial biomass does not increase at the same rate as that of the phytoplankton is currently unexplained, although hypotheses consistent with recent literature results can be advanced for test. Our ability to predict bacterial abundance from chlorophyll data need not be an end in itself, but provides an opportunity to pose more specific questions that should help to reduce the scatter about the line of best fit, and to gain insight into the role that bacteria play in aquatic environments.

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Evolution de la variabilité génétique inter et intra-populations de populations de Salmonidés issues de mêmes pools géniques

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L'analyse électrophorétique de 14 systèmes enzymatiques correspondant à 31 loci a permis d'étudier l'évolution de la variabilité inter et intra-populations de populations issues d'un même pool génique initial au cours de six générations. Nous avons examiné quatre populations d'Omble de fontaine (*Salvelinus fontinalis*) et cinq de Truite fario (*Salmo trutta*) prélevées aux îles Kerguelen où des introductions contrôlées de Salmonidés ont été réalisées. Le taux d'hétérozygotie de ces populations reste élevé et est équivalent aux niveaux de variabilité observés au cours d'autres études de polymorphisme portant sur les mêmes espèces. Dans chaque espèce, le schéma de différenciation génétique est cohérent avec la « proximité hydrographique » des populations et avec l'origine génétique. Ainsi, à l'intérieur d'un même pool initial, les dendrogrammes regroupent en premier lieu les différents écotypes prélevés dans un même système hydrographique, qui semblent donc résulter d'une variabilité comportementale au sein de populations panmictiques. Enfin, il paraît illusoire d'établir des relations temps–distance génétique sur la seule base de données électrophorétiques.

Through electrophoretic analysis of 14 enzymatic systems corresponding to 31 loci, we studied the change over six generations in the intra- and inter-population variability of salmonid populations from the same initial genetic pool. Four collections of brook trout (*Salvelinus fontinalis*) and five of brown trout (*Salmo trutta*) from recently acclimatized stocks in the Kerguelen Islands were examined. The heterozygosity level of these populations remained high and corresponded to the levels of variability observed in other polymorphism studies of the same species. In each species, there was a consistent pattern of genetic differentiation with the "hydrographic proximity" of the populations and with the genetic origin. Within one and the same initial genetic pool, the dendrograms grouped primarily the different ecotypes collected in the same hydrographic system. Thus, these ecotypes seem to result from a behavioral variability within a panmictic population. Finally, establishment of time–genetic distance relationships on the sole basis of electrophoretic data appears unwarranted.

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Aux îles Kerguelen (Territoire des Terres Australes et Antarctiques Françaises), des tentatives d'acclimatation de salmonidés ont eu lieu ces dernières années. Celles concernant la truite fario (*Salmo trutta*) et l'omble de fontaine (*Salvelinus fontinalis*) ont abouti puisque plusieurs cycles reproductifs ont été obtenus; pour les essais concernant le saumon atlantique (*Salmo salar*), le diagnostic est encore réservé car les premières reproductions naturelles ont seulement lieu actuellement (Davaine et Beall 1982a).

Nous avons entrepris une étude de la structure génétique de ces différentes populations de salmonidés qui se sont développées dans des rivières vierges de poissons. Cette caractérisation génétique devait permettre de vérifier le niveau de variabilité existant dans ces populations, compte tenu d'une part des risques d'introduction d'une base génétique étroite (peu de variabilité génétique dans l'effectif de départ du fait de l'utilisation possible d'un faible nombre de géniteurs pour produire les lots d'œufs servant aux alevinages), d'autre part des risques de pertes de variabilité génétique, par sélection ou dérive génétique, dans les premières phases de colonisation, enfin de l'absence totale de flux génique exogène. Elle devait permettre également d'étudier une situation originale de différenciation d'un stock génétique placé dans différents milieux et en particulier de voir s'il existait une relation entre différenciation génétique et différenciation en écotypes. En effet, dans les cas des truites fario d'origine « Bidarray »[1] et des ombles de fontaine, d'une part un même lot d'œufs a servi à l'alevinage de plusieurs systèmes hydrographiques et d'autre part, les populations ont ségrégé en plusieurs écotypes (« rivière », « mer » ou « lac » pour la truite fario; « rivière » ou « lac » pour l'omble de fontaine) (tableau 1; fig. 1).

Nous avons étudié le polymorphisme de 14 systèmes protéi-

[1]Pisciculture du Sud-Ouest de la France.

ques correspondant à 31 loci sur cinq échantillons de truite fario et quatre échantillons d'omble de fontaine.

Matériels et méthodes

Le tableau 1 reproduit l'historique des populations étudiées ainsi que les principales caractérisques des milieux dans lesquels elles furent implantées. Les échantillons ont été prélevés au cours de l'année 1981 par pêche électrique. Les tailles des échantillons analysés sont indiqués au tableau 1. Les tissus prélevés ont été conservés à −30°C jusqu'à l'analyse.

Les conditions d'extraction, de migration et de révélation ont été décrites dans des publications antérieures (Guyomard et Krieg 1983). Les systèmes étudiés sont *AAT* (aspartate aminotransférase), *ADH* (alcool déhydrogénase), *AGP* (α-glycérophosphate déhydrogénase), *CPK* (créatine phosphokinase), *IDH* (isocitrate déhydrogénase), *LDH* (lactate déhydrogénase), *MDH* (malate déhydrogénase), *ME* (malic enzyme), *6PGDH* (6-phosphogluconate déhydrogénase), *PGM* (phosphoglucomutase), *PGI* (phosphoglucose isomérase), *PMI* (phosphomannose isomérase), *SDH* (sorbitol déhydrogénase) et *SOD* (superoxide dismutase). La nomenclature des loci et allèles est celle proposée par May (1980). Nous avons considéré qu'un variant génétique ayant une mobilité similaire à celle d'un allèle déjà cité dans la littérature était identique à cet allèle.

Les taux d'hétérozygotie et les distances génétiques ont été estimés respectivement par la diversité génique et la distance standard définies par Nei (1972). L'hétérozygotie totale (H_T) a également été définie précédemment (Guyomard 1981).

Résultats

Le polymorphisme de 14 systèmes protéiques représentant 31 loci a été examiné dans chaque population de truite fario et d'omble de fontaine. Les variations observées et leurs interprétations génétiques sont conformes à celles décrites dans les études précédentes de populations naturelles (Allendorf et al. 1976, 1977 ; Taggart et al. 1981 ; McGlade et McCrimmon 1979 ; Ryman et Stahl 1981 ; Stoneking et al. 1981 ; Guyomard et Krieg 1983) et de ségrégation des gènes (May et al. 1979a, 1979b ; Guyomard et Krieg 1983).

Dans le cas de la truite fario, les allèles observés s'identifient facilement avec ceux qui ont été déjà décrits dans la littérature. Dans le cas de l'omble de fontaine, les comparaisons s'avèrent impossibles, parce que certains loci très polymorphes dans les études précédentes (Stoneking et al. 1981) sont fixés, dans nos populations, pour un allèle que l'absence de variations ne permet pas de définir (cas de Agp-2, Cpk-2, Idh-3, Ldh-3, Pgm-2).

Les fréquences observées dans les populations de truite fario et d'omble de fontaine sont indiquées au tableau 2. Chez l'omble de fontaine, les loci Aat-1 et Aat-2 possèdent en commun les deux allèles 100 et 118. Nous avons donc admis que la fréquence de l'allèle 100 (et par conséquent, celle de l'allèle 118) est la même aux deux loci, hypothèse qui minimise la distance inter populations pour Aat-1 et 2 (Guyomard 1981). Cette hypothèse est compatible avec l'équilibre de Hardy–Weinberg à ces deux loci.

Les déviations par rapport aux proportions de Hardy–Weinberg ont été également calculées pour chaque locus et chaque échantillon respectant les conditions d'application du test de χ^2 (nombres théoriques ≥ 3 dans chaque classe). Des

TABLEAU 1. Caractéristiques des échantillons étudiés.

Système hydrographique	Espèce	Date et effectif de fondation de la population	Origine des lots d'œufs d'alevinage	Ecotype	Abbréviation désignant l'échantillon	Effectif de l'échantillon
Val Studer	*Salmo trutta*	721 truitelles (1+) en 1959 2 000 alevins (?+) en 1961	Pisciculture de Bidarray (Pyrénées-Atlantiques) (lots d'œufs de 1958 et 1961)	Lac } Isolés reproductivement par l'existence de cascades infranchissables Mer }	TS TMS	15 11
	Salvelinus fontinalis	2 000 alevins (0+) en 1961	Pisciculture de Lées-Athas (Pyrénées-Atlantiques)	Lac	OLS	20
				Poissons pris en rivière mais à l'amont des cascades, donc pouvant provenir de la reproduction des ombles écotype lac	ORS	40
Rivière du Chateau	*Salmo trutta*	22 truites (4+) en 1962	Pisciculture de Bidarray (lot d'œufs de 1958)	Mer	TMch	40
				Rivière	Tch	44
	Salvelinus fontinalis	500 alevins (0+) en 1961	Pisciculture de Lées-Athas	Rivière	OC	41
Val de l'Acaena	*Salmo trutta*	21 000 alevins (0+) en 1979	Pisciculture polonaise utilisant un stock de géniteurs de truite de mer de la rivière Slupia	Juvéniles (cycle reproductif non encore bouclé)	TVA	20
Rivière du Sud	*Salvelinus fontinalis*	2 000 alevins (0+) en 1961 48 ombles (1+) en 1962	Pisciculture de Lées-Athas	Rivière	OS	43

FIG. 1. Principaux cours d'e

cours d'eau peuplés (partie Est de Kerguelen).

TABLEAU 2. Fréquences alléliques de 14 locus polymorphes et tests de χ^2 de déviation par rapport aux proportions de Hardy–Weinberg pour cinq échantillons de truite fario et quatre échantillons d'omble de fontaine (*$P \leq 0.05$; **$P \leq 0.01$).

	Truites					Ombles			
Système polymorphe	TMCh (40)	TCh (44)	TMS (11)	TS (15)	TVA (20)	OC (41)	OS (43)	OLS (20)	ORS (40)
$AAT_{1,2}$ 100	1,00	1,00	1,00	0,86	0,925	0,69	0,71	0,68	0,64
140(S.t) ou 118(S.f)	0	0	0	0,14 (14)	0,075	0,31 (40)	0,29	0,32 (19)	0,36
						1,24			2,35
AAT_4 100	0,64	0,63	0,86	0,90	0,75				
74	0,36	0,37 (43)	0,14	0,10	0,25				
	0,252	0,004			4,355*				
ADH −100						0,83	0,66	0,66	0,71
0						0,16	0,30	0,175	0,14
−205						0,01	0,04	0,175	0,15
χ_1^2 (calculé en regroupant −205 et 0)							4,571*		
CPK_1 100	0,75	0,71	0,59	0,50	0,875				
115	0,25	0,29 (42)	0,41	0,50 (14)	0,125				
		1,396		0,284					
AGP_2 100	0,86	0,795	0,77	0,71	0,925	0,78	0,67	0,60	0,576
50(S.t.) ou 78(S.f.)	0,14	0,205	0,23	0,29 (14)	0,075	0,22	0,33	0,40	0,425
						0,874	0,156	0,034	0,023
IDH_3 100	0,45	0,62	0,73	0,50	0,975				
160	0,55 (38)	0,38 (26)	0,27	0,50 (14)	0,025				
	0,158	0,932		0,285					
IDH_4 100						0,66	0,87	0,95	1,00
lent						0,34	0,13 (41)	0,05	0
						2,973			
LDH_4 100						0,73	0,65	0,72	0,71
72						0,27	0,35	0,28	0,29
						0,570	0,263		1,026
MDH_2 100	0,66	0,78	0,68	0,63	0,80				
152	0,34	0,22 (43)	0,32	0,37	0,20				
		0,102							
$MDH_{3,4}$ 100	0,725	0,77	0,77	0,82	0,775	0,80	0,88	0,85	0,72
80(S.t.)	0,275	0,23	0,23	0,18 (14)	0,225				
120(S.f.)						0,20	0,12	0,15	0,28
	2,520					1,987			
ME_1 100						0,60	0,73	0,60	0,55
0						0,40	0,27	0,40	0,45
						0,056	0,007	0,556	1,799
6PGDH 100	0,96	1,00	1,00	1,00	1,00				
80	0,04	0	0	0	0				
SDH_1 0	0,68		0,59	0,68	0,825				
100	0,32 (36)		0,41	0,32 (11)	0,175				
	0,263								

déviations significatives n'ont été mises en évidence que dans deux cas (tableau 2). Les populations sont donc en équilibre panmictique.

Nous avons également testé l'existence de différences significatives des fréquences alléliques entre populations. Dans le cas de fréquences proches de 1 et d'effectifs théoriques faibles ($n \leq 3$), nous avons appliqué le test exact (Lamotte 1957). Des différences significatives au seuil de 5 % ont été observées dans 20 cas sur 90 chez la truite fario (tableau 3) et 13 cas sur 42 chez l'omble de fontaine (tableau 3). On peut donc conclure à l'hétérogénéité génétique des deux groupes.

Les taux d'hétérozygotie et distances génétiques ont été calculés sur 29 loci chez la truite fario (*PMI* et *SDH* non inclus, tableau 4) et 31 loci chez l'omble de fontaine (*SDH* codé par un seul locus chez cette espèce, tableau 4). Les taux d'hétérozygotie sont voisins dans chaque groupe de populations issues d'un même pool génique : 8 à 9 % chez la truite fario (9 à 10 % si *PMI* est inclus), 8 % chez l'omble de fontaine. La population du val

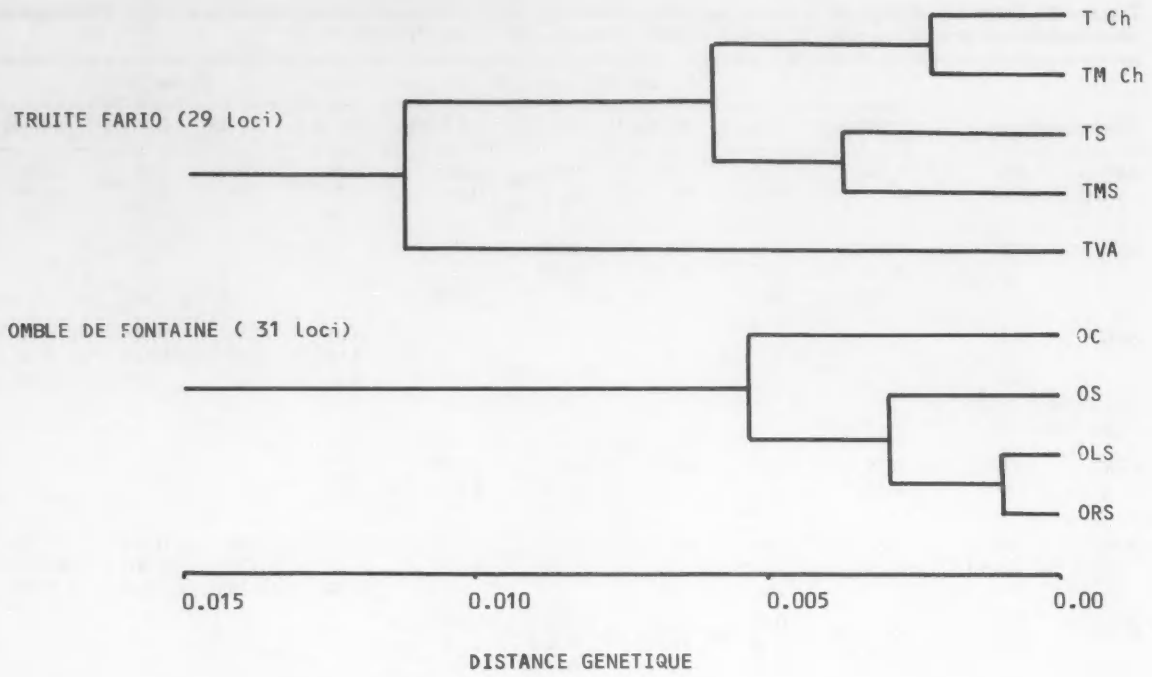

FIG. 2. Dendrogramme des distances génétiques.

TABLEAU 3. Loci dont les fréquences sont significativement différentes dans les échantillons de truite fario et d'omble de fontaine (entre parenthèses) des îles Kerguelen (*P ≤ 0,05 ; **P ≤ 0,01 ; ***P ≤ 0,001).

	TMCh (OS)	TCh (OLS)	TMS (ORS)	TS
TMS (OC)	*IDH-3** *AAT-4** *(ADH** *IDH-4***)	*AAT-4** *(ADH*** *AGP-1** *IDH-4****)	*(ADH** *IDH-4*** *AGP-1***)	
TS (OS)	*AAT-4*** *AAT-1, 2*** *CPK-1**	*AAT-4*** *AAT-1, 2*** *CPK-1** *(ADH**)	*(ADH*** *IDH-4**** *MDH-3, 4*** *ME-1**)	
TVA	*AAT-1, 2** *IDH'3****	*AAT-1, 2*** *CPK-1, 2** *IDH-3****	*CPK-1** *SDH-1** *IDH-3****	*CPK-1**** *AGP-2** *IDH-3****

TABLEAU 4. Distances génétiques standards entre les échantillons de truite fario et d'omble de fontaine (entre parenthèses). Les taux d'hétérozygotie sont sur les diagonales (résultats sur 29 loci pour la truite fario et 31 pour l'omble de fontaine).

	TMCh (OC)	TCh (OS)	TMS (OLS)	TS (ORS)	TVA
TMCh	0,087				
(OC)	(0,087)				
TCh	0.0023	0,082			
(OS)	(0,0039)	(0,084)			
TMS	0,0066	0,0033	0,078		
(OLS)	(0,0053)	(0,0018)	(0,086)		
TS	0,0069	0,0073	0,0038	0,091	
(ORS)	(0,0066)	(0,0040)	(0,0009)	(0,088)	
TVA	0,0127	0,0071	0,0074	0,0178	

de l'Acæna, d'origine polonaise, s'individualise à la fois par son taux d'hétérozygotie (5,5 %, tableau 4) et ses distances génétiques par rapport aux autres populations (tableau 4 ; fig. 2). Les χ^2 cumulés ne mettent en évidence aucune différence significative entre TMS et TS d'une part et TMCh et TCh d'autre part. Les différences entre les trois groupes TCh-TMCh, TS-TMS et TVA chez la truite fario, ORS-ORL, OS et OC chez l'omble de fontaine sont significatives.

Discussion

Variabilité intra-population

Les niveaux de variabilité intra-population observés sont élevés. Dans le cas de la truite fario, les taux d'hétérozygotie se maintiennent à des niveaux équivalents à ceux des populations de pisciculture (Guyomard et Krieg 1983 ; F. Krieg et R. Guyomard, données non publiées). Dans le cas de l'omble de fontaine, les valeurs observées (0,08) correspondent à celles des populations naturelles (Stoneking et al. 1981), mais restent néanmoins très inférieures à la variabilité totale de l'espèce (H_T = 0,148, Stoneking et al. 1981) et donc à la variabilité théorique possible dans les souches d'élevage.

Les niveaux de variabilité sont remarquablement homogènes dans chaque groupe de populations. Ces hauts niveaux de variabilité intra population, ainsi que leur stabilité dans chaque groupe plaident en faveur d'une bonne conservation de la variabilité génétique initiale. Sous l'hypothèse de neutralité, la variabilité du pool génique d'origine peut être estimée par H_T

(truite fario : 0,100 ; omble de fontaine : 0,087) et comparée à la valeur moyenne actuelle du taux d'hétérozygotie (0,095 chez la truite fario, population du val d'Acæna non incluse ; 0,086 chez l'omble de fontaine). La perte de variabilité est faible (5 % de H_T chez la truite fario ; 1 % chez l'omble de fontaine) et il ne semblerait pas y avoir eu d'effet fondateur sur la variabilité intra population. Les données de dynamique des populations, indiquant une croissance continue de la taille des populations, vont dans ce sens Davaine et Beall 1982b).

Variabilité inter-populations

Les distances génétiques sont cohérentes, d'une part avec la « parenté hydrographique » des échantillons, et d'autre part avec la constitution génétique initiale. Ainsi le dendrogramme regroupe d'abord les différents écotypes prélevés dans une même rivière, qu'il s'agisse de l'omble de fontaine (couple ORS/OLS) ou de la truite fario (TS/TMS et TMCh/TCh). Les divers écotypes observés semblent donc résulter d'une variabilité comportementale au sein de populations totalement ou partiellement panmictiques ; cette interprétation, qui ne présume en rien de l'existence d'une base génétique de cette variabilité comportementale, est tout à fait compatible, d'une part avec l'origine génétique unique des populations considérées (TS, TMS, TCh, TMCh et ORS, OLS) et d'autre part avec le brassage génétique généralement pratiqué en pisciculture. Enfin, dans le cas de la truite fario, la population du val de l'Acæna, issue d'une autre pisciculture, se différencie plus nettement.

Les différenciations génétiques dues à cinq ou six générations d'isolement reproducteur sont équivalentes dans les deux espèces (truite fario : 0,7 % ; omble de fontaine : 0,65 %). Les deux populations fondatrices n'ont évidemment pas pu être examinées et leurs contreparties actuelles n'ont que peu de valeur vis-à-vis de cette étude car, depuis, elles ont reçu des apports extérieurs en provenance d'autres pisciculture. Cependant, dans le cas de la truite fario, nos résultats (Guyomard et Krieg 1983 ; F. Krieg et R. Guyomard, données non publiées) montrent que la différenciation entre populations de pisciculture et les échantillons TS, TMS, TCh, TMCh est faible (distance moyenne : 0,006 ; valeurs extrêmes : 0,003–0,010). Ceci est cohérent avec l'hypothèse d'une souche fondatrice ayant un « profil électrophorétique » très proche de ceux des autres populations d'élevage (qui forment elles-mêmes un groupe très homogène) et accrédite à nouveau l'idée d'une très faible perte de la variabilité initiale dans les souches TS, TMS, TCh et TMCh.

L'une des thèses les plus importantes issues du développement de la biologie et de la taxonomie moléculaires, celle de « l'horloge évolutive », propose que l'évolution des protéines se ferait à un taux constant (Sarich et Wilson 1966 ; Nei 1975 ; Wilson et al. 1977 ; Kimura 1979). Cette constance du taux d'évolution peut être démontrée de façon théorique si l'on admet la neutralité de la variabilité moléculaire : dans ce cas, la différenciation génétique ne dépend que du taux de mutation et devient donc une fonction linéaire du temps si les taux de mutation sont constants sur la période considérée (Ewens 1979). Cette thèse a parfois été utilisée pour établir le temps de divergence à partir du taux de différenciation entre populations. Cependant, sous l'hypothèse de neutralité, les différenciations génétiques que nous avons observées dans cette étude résultent principalement d'une dérive génétique et dépendent donc surtout de la taille effective de chaque population. Même si la différenciation génétique s'avérait être en relation linéaire avec le temps sur de longues périodes, il nous paraît évident que cette relation doit être profondément altérée par la dérive aléatoire pour de faibles nombres de générations. Il est fort probable que cette remarque, qui s'applique à la situation décrite dans cet article, tienne également pour d'autres études de populations naturelles et des distances génétiques plus importantes. En conséquence, il nous paraît illusoire de vouloir établir des « horloges évolutives » sur la seule base de données électrophorétiques intraspécifiques, donnant généralement des niveaux de différenciation génétique peu élevés.

Enfin, sur un plan plus pratique, celui de la gestion des populations naturelles, nous émettons l'hypothèse qu'il n'est pas nécessaire de prendre en considération des niveaux de différenciation équivalents à ceux que nous avons observés, dans la mesure où ils sont susceptibles de s'établir en des temps relativement courts.

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Bioavailability of Pb and Zn from Mine Tailings as Indicated by Erythrocyte δ-Aminolevulinic Acid Dehydratase (ALA-D) Activity in Suckers (Pisces: Catostomidae)

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The activity of the erythrocyte enzyme δ-aminolevulinic acid dehydratase (ALA-D) was measured in 35 catostomids (black redhorse, *Moxostoma duquesnei*; golden redhorse, *M. erythrurum*; northern hogsucker, *Hypentelium nigricans*) collected from three sites on a stream contaminated with Pb-, Cd-, and Zn-rich mine tailings and from an uncontaminated site upstream. Enzyme activity was expressed in terms of hemoglobin (Hb), DNA, and protein concentrations; these variables can be determined in the laboratory on once-frozen blood samples. Concentrations of Pb and Zn in blood and of Pb in edible tissues were significantly higher, and ALA-D activity was significantly lower, at all three contaminated sites than upstream. At the most contaminated site, ALA-D activity was 62–67% lower than upstream. Lead concentrations in the edible tissues and in blood were positively correlated ($r = 0.80$), whereas ALA-D activity was negatively correlated with Pb in blood ($r = -0.70$) and in edible tissues ($r = -0.59$). Five statistically significant relations between Pb and Zn in blood and ALA-D activity were determined. The two models that explained the highest percentage (>74%) of the total variance also included factors related to Hb concentration. All five significant models included negative coefficients for variables that represented Pb in blood and positive coefficients for Zn in blood. The ALA-D assay with results standardized to Hb concentration represents an expedient alternative to the more traditional hematocrit standardization, and the measurement of ALA-D activity by this method can be used to document exposure of fish to environmental Pb.

On a quantifié l'activité de l'enzyme érythrocytaire acide lévulique δ-amine déshydratase (ALA-D), chez 35 catostomidés (suceur noir, *Moxostoma duquesni* ; suceur doré, *M. erythrurum* ; meunier à tête carrée, *Hypentelium nigricans*) capturés en trois endroits d'un ruisseau contaminé par des stériles miniers riches en Pb, Cd et Zn et en un endroit non contaminé, en amont. L'activité enzymatique est exprimée selon les concentrations d'hémoglobine (Hb), d'ADN et de protéines, variables qui peuvent être déterminées en laboratoire sur des échantillons de sang congelés. Les concentrations de Pb et de Zn dans le sang et de Pb dans les tissus comestibles étaient significativement plus élevées et l'activité de l'ALA-D était significativement plus faible aux trois sites contaminés qu'en amont. Au site le plus contaminé, l'activité de l'ALA-D était de 62 à 67 % plus faible qu'en amont. Les concentrations de plomb dans les tissus comestibles et le sang étaient en corrélation positive ($r = 0,80$) tandis que l'activité de l'ALA-D était en corrélation négative avec le Pb dans le sang ($r = 0,70$) et dans les tissus comestibles ($r = 0,59$). On a trouvé cinq relations statistiquement significatives entre le Pb et le Zn dans le sang et l'activité de l'ALA-D. Les deux modèles qui expliquaient le plus haut pourcentage (>74 %) de la variance totale comprenaient aussi des facteurs reliés à la concentration de Hb. Les cinq modèls significatifs englobaient des coefficients négatifs du Pb sanguin et des coefficients positifs du Zn sanguin. L'analyse de l'ALA-D avec résultats normalisés en fonction de la concentration de Hb représente une solution pratique comparativement à la traditionnelle normalisation de l'hématocrite. La quantification de l'activité de l'ALA-D à l'aide de cette première méthode peut être utilisée pour analyser l'exposition du poisson au Pb environnemental.

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The erythrocyte enzyme δ-aminolevulinic acid dehydratase (ALA-D, E.C. 4.2.1.24) catalyzes the formation of porphobilinogen (PBG), a hemoglobin (Hb) precursor, from aminolevulinic acid (Finelli 1977). This enzyme's activity is highly sensitive to Pb; inhibition in humans typically occurs at lower blood-Pb concentrations and earlier than other symptoms of lead poisoning (Secchi et al. 1974). Because it is also relatively easy to measure, ALA-D activity has long been used to diagnose Pb exposure in humans. More recently, this assay has been used to detect lead poisoning (from lead shot ingestion) in waterfowl (e.g. Dieter 1979).

The activity of ALA-D in fish blood is also depressed by Pb. Hodson et al. (1977) showed that the inhibition of erythrocyte ALA-D is specific for Pb, that reduction in activity occurs rapidly and can be detected at exposure concentrations near the "no effect" level, and that the enzyme system appears to be present in many fishes. They also concluded that the assay for blood ALA-D activity could be used as a "short-term indicator

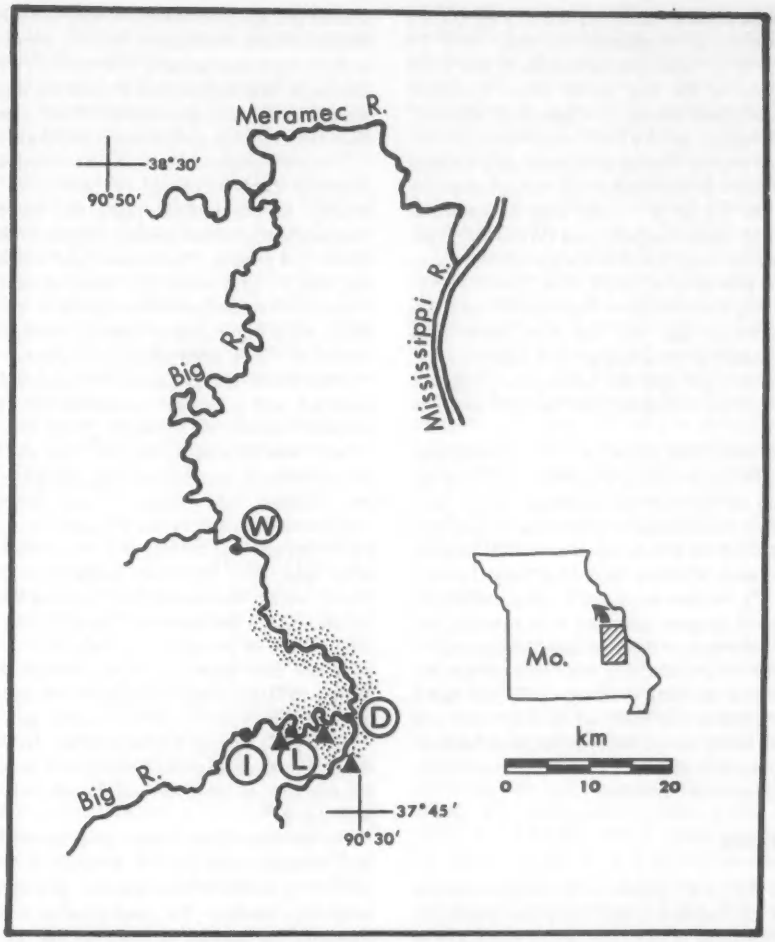

FIG. 1. Big River study area. Shown are three major sources of mine tailings to the river (triangles), river reaches where the sediments are composed primarily of tailings (shaded area), and collection sites at Irondale (I), Leadwood (L), Desloge (D), and Washington State Park (W).

of long-term sublethal effects of lead on fish." Our objective was to adapt the ALA-D assay for use in the field and use it to document the biological availability and biochemical activity of Pb and Zn in a river system contaminated by mine tailings.

Study Area and Background

The Big River drains Missouri's "Old Lead Belt" (Fig. 1), an area that formerly led the world in lead production (U.S. Geological Survey 1967). Although the last mine in the Old Lead Belt was closed in 1972, tailings from past mining and ore processing activities are present throughout the district. Lead concentrations in these tailings range from about 1000 $\mu g \cdot g^{-1}$ to more than 4000 $\mu g \cdot g^{-1}$ (dry weight), varying with location and particle size distribution (Novak and Hasselwander 1980; Schmitt and Finger 1982). The tailings, which are also rich in Cd and Zn, have entered the Big River at several locations and constitute a high percentage of the bottom sediments in some reaches (Whitley 1979). Kramer (1976) estimated that some 227 Mt of tailings were produced during the 100 yr of commercial mining in the Old Lead Belt. About 100 Mt remain in eight discrete locations as unvegetated chat piles and tailings ponds (Missouri Department of Conservation and U.S. Army Corps of Engineers, St. Louis District, unpubl.). The fate of the other 117 Mt is unknown; the material was widely distributed by the mining enterprises for use as agricultural lime, and considerable wind and water erosion has taken place at the tailings deposits (Whitley 1979).

Erosion of mine tailings into the Big River was common knowledge. However, evidence that aquatic biota were contaminated did not become available until 1979 (T. May, U.S. Fish and Wildlife Service, pers. comm.). Lead concentrations of 18.0 $\mu g \cdot g^{-1}$ (wet weight) in longear sunfish (*Lepomis megalotis*), 14.5 $\mu g \cdot g^{-1}$ in black redhorse (*Moxostoma duquesnei*), and 9.0 $\mu g \cdot g^{-1}$ in smallmouth bass (*Micropterus dolomieui*) were reported in samples of whole fish collected 25 km downstream of the tailings deposits. These levels were almost

threefold greater than the highest values reported by the (U.S.) National Pesticide Monitoring Program for the years 1976–77 (May and McKinney 1981). Lead concentrations in fish from uncontaminated reaches of the Big River never exceeded $0.25\ \mu g \cdot g^{-1}$. Results of a later survey (J. Czarneski, Missouri Department of Conservation, pers. comm.) showed that concentrations in the edible tissues of some taxa, especially suckers (Catostomidae), from areas downstream of the tailings deposits frequently exceeded the $0.3\ \mu g \cdot g^{-1}$ (wet weight) guideline established by the World Health Organization (WHO 1972) for Pb in foods. Consequently, local fishermen were warned not to eat large quantities of fish from affected areas (Shirk 1980). Spokesmen for the mining interests immediately challenged this precaution on the grounds that the fish may have been contaminated during handling, processing, and analysis (e.g. Settle and Patterson 1980), and that the metals were probably sorbed onto the outside of the fish rather than incorporated into the tissues.

In our study, we collected both blood and edible tissues and analyzed the blood for Pb and for ALA-D activity to (1) test the feasibility and validity of the enzyme technique under field conditions and (2) either substantiate or refute the presence of elevated Pb in suckers from the Big River. Because the amount of Zn in the blood may also influence ALA-D activity (Hodson et al. 1977; Finelli 1977), we also measured Zn concentrations. We contended that blood samples collected with a needle and syringe and analyzed according to the procedures described by Patterson and Settle (1976) are relatively free from contamination during collection and handling (Wiener 1982) and that a statistically definable relation between ALA-D activity and metal concentrations in blood would demonstrate both biological availability and biochemical activity of Pb derived from mine tailings in the aquatic environment.

Materials and Methods

We collected black redhorse, golden redhorse (*Moxostoma erythrurum*), and northern hogsuckers (*Hypentelium nigricans*) in April 1981 by electrofishing at four sites on the Big River: at an uncontaminated site about 5 km upstream from the tailings deposits, near Irondale; at two sites (Leadwood and Desloge) within the tailings area; and at Washington State Park, about 59 km farther downstream (Fig. 1). We attempted to capture 10 adult black redhorse of similar size at each site; however, at some sites the sample had to be augmented with golden redhorse and northern hogsuckers, and electrofishing at Leadwood and Desloge yielded only these latter species. Fish were kept alive in tubs of river water and then anesthetized by immersion in tricaine methanesulfonate (MS-222) for several minutes before blood and tissue samples were collected. After each anesthetized fish was weighed and measured (total length, TL), a sterile, disposable 3-mL syringe equipped with a 20-gauge needle was rinsed with a $1760\text{-unit(USP)} \cdot mL^{-1}$ solution of sodium heparin and filled to capacity with blood drawn by caudal peduncle puncture. The blood was then dispensed from the syringe into sterile, volumetrically calibrated, disposable borosilicate glass culture tubes (15 × 85 mm) that we had prepared earlier in the following manner. First, each culture tube was calibrated by adding 1.0 mL of distilled water and scribing a mark at the bottom of the meniscus. The tubes were then cleaned by submersion first in $16\ mol \cdot L^{-1}\ HNO_3$ and then in $12\ mol \cdot L^{-1}$ HCl, rinsed in ultrapure H_2O ($15–18\ \mu\Omega \cdot cm^{-1}$), and oven-dried at 125°C. The ribbed polyethylene caps were soaked in a mixture of 40% HNO_3 and 20% HCl for at least 3 d and then rinsed in ultrapure H_2O. To prevent clotting, 250 μL of sodium heparin solution ($1760\text{ unit(USP)} \cdot mL^{-1}$) was dispensed into each tube and swirled to coat the entire inside. The tubes were then dried in an oven at 125°C, removed, and sealed in polyethylene bags until needed for blood collection.

Two subsamples, dispensed into separate culture tubes, were prepared form the blood of each fish. The first, dispensed exactly to the scribed mark on the tube, was used for volumetrically based measurements of ALA-D activity, Hb, DNA, and protein. The second tube was filled to near capacity and used for gravimetrically based measurements of Pb and Zn concentrations and moisture content. After blood collection, the tubes were immediately capped, frozen in dry ice, and kept frozen at −23°C until analysis (<24 h).

After blood was collected, we sampled the edible tissues of each fish with a method commonly used by local fishermen to prepare suckers for cooking. Each fish was scaled; then a "fillet," which included the skin, ribs, and intermuscular bones, was removed from the right side, placed in a clean polyethylene bag, chilled, and frozen. These samples were sent to a commercial laboratory for Pb analysis, where the fillets were partly thawed and cut into 0.5-cm cubes. The cubes from each fillet were then randomly sampled to provide 2.5–3.5 g of tissue, which was completely thawed and homogenized in a blender. Each homogenized sample was digested by heating with 25 mL of $16\ mol \cdot L^{-1}\ HNO_3$ in a 100-mL Kjeldahl flask over low heat until only 1 mL remained. This digestate was diluted to 50 mL with deionized water and analyzed for Pb with a Perkin-Elmer model 305-B[1] atomic absorption spectrophotometer (AAS) equipped with an HGA-2100 graphite furnace and deuterium arc background correction. Results were reported as micrograms per gram wet weight, with a lower detection limit of $0.05\ \mu g \cdot g^{-1}$.

For our field study, it was necessary to modify the technique that Hodson et al. (1977) developed for measuring ALA-D activity in rainbow trout (*Salmo gairdneri*) exposed to Pb in laboratory studies. We used smaller volumes of blood, and standardized enzyme activity to Hb, DNA, and protein (= specific enzymatic activity) concentrations. ALA-D activity has traditionally been reported in units of nanomoles of product formed per millilitre of red blood cells (RBC); however, hematocrit determinations and RBC counts are difficult to perform in the field and require fresh blood. The blood variables we measured can be determined on once-frozen blood samples in the laboratory.

The 1-mL blood samples were prepared for analysis of ALA-D activity, Hb, DNA, and protein as follows. The samples were thawed at room temperature only until they could be removed from the culture tubes. Each sample was then transferred to a chilled grinding tube, and 6 mL of $0.15\ mol \cdot L^{-1}$ KCl buffer solution was added. The tube was transferred to an ice bath and the mixture was homogenized with a Wheaton Instruments grinder equipped with a Teflon pestle.

We adapted the Hb assay described by Natelson (1971), designed for whole blood, for use with blood homogenates. We added 5 mL of pH 2 KCl–HCl buffer to 200 μL of homogenate and allowed the mixture to incubate at room temperature for 5 min. This mixture's absorbance was read at 540 nm with a Bausch & Lomb Spectronic 100 spectrophotometer against a

[1]Reference to trade names does not imply Government endorsement of commercial products.

buffer blank and compared with a standard curve prepared from a solution of bovine Hb (Sigma Chemical). Results were reported as milligrams Hb per millilitre.

We measured protein content with a slight variation of the procedure outlined by Mattenheimer (1970), beginning by mixing 3 mL of freshly prepared Na_2CO_3–$CuSO_4$ (1 mL of 0.5% $CuSO_4$ (0.5 g of $CuSO_4$ in 50 mL H_2O, buffered with 1 g of $Na_3C_6H_5O_7 \cdot 2H_2O$, diluted to 100 mL with H_2O) in 50 mL of 2% Na_2CO_3 (5 g of Na_2CO_3 in 250 mL of 0.1 mol·L^{-1} NaOH)) with 20 μL of blood homogenate. After allowing the mixture to stand at room temperature for 10 min, we added 100 μL of 1.0 mol·L^{-1} Folin reagent and read the sample's absorbance at 600 nm against a blank containing 20 μL of KCl buffer. The measured absorbance was compared with a standard curve prepared from a solution of 200 mg of bovine albumin (Sigma Chemical) in 25 mL of KCl buffer. Results were reported as milligrams protein per millilitre.

We slightly modified the technique outlined by Schneider (1957) to measure DNA. To 600 μL of blood homogenate, we added 2 mL of 10% trichloroacetic acid (TCA), mixed and centrifuged (Beckman TJ-6) the sample, and aspirated the supernatant. Another 2 mL of 10% TCA was added, and the sample was again centrifuged and aspirated. The remaining solids were washed twice by adding 2 mL of 95% ETOH, and centrifuged and aspirated after each wash. We added 1.5 mL of 5% TCA to the remaining material. This mixture was incubated at 90°C for 30 min and centrifuged, and 1 mL of the supernatant was transferred to a clean test tube. Into this solution we mixed 2 mL of diphenylamine (DPA, (Mallinkrodt)) reagent (1 g of DPA in 100 mL of 17.4 mol·L^{-1} HAc, 2.75 mL of 16 mol·L^{-1} H_2SO_4, and 0.5 mL of aqueous acetaldehyde (320 mg of acetaldehyde–sodium bisulfite (Eastman-Kodak) in 20 mL of H_2O)). After incubation at 100°C for 30 min, we read the mixture's absorbance at 600 nm against a 5% TCA blank and compared it with a standard curve prepared from a solution of 10 mg of salmon DNA (sodium salt, Sigma Chemical) in 100 mL of 5% TCA. Results were reported as milligrams DNA per millilitre.

For the ALA-D assay, we added 100 μL of δ-aminolevulinic acid (ALA) reagent (34 mg of ALA (Sigma Chemical) + 25 mL of 0.5% Triton X-100 (1 mL of Triton X-100 in 200 mL of 0.1 mol·L^{-1} phosphate buffer)) to 200 μL of blood homogenate. After incubation at 37°C for 1 h, 300 μL of TCA–$HgCl_2$ (4.0 g of TCA + 2.7 g of $HgCl_2$ in 50 mL of H_2O) was added, and the mixture was centrifuged. To 400 μL of the supernatant we added 350 μL of freshly prepared Ehrlich's reagent (175 mg of $HgCl_2$ + 1.0 g of *p*-dimethylaminobenzaldehyde (PAB) in 42 mL of 17.4 mol·L^{-1} HAc, 10 mL of 11.7 mol·L^{-1} $HClO_4$, and 3 mL of H_2O). After incubation for 15 min at room temperature, the mixture's absorbance was measured at 553 nm against a blank containing 100 μL of ALA, 200 μL of KCl, and 2 mL of TCA–$HgCl_2$ and compared with a standard curve prepared from a solution of 10 μg of PBG in 1.32 mL of KCl buffer solution, 68 mL of Triton X-100, and 2 mL of TCA– $HgCl_2$. Results were reported in enzyme units (nmol·L^{-1} PBG·mL-h^{-1}).

The culture tubes with blood to be analyzed for Pb and Zn residues were wiped clean with acetone, uncapped, and weighed. The samples were then lyophilized in a Virtis freeze drier and reweighed. To digest the sample, we added 1 mL of 16 mol·L^{-1} HNO_3 to each tube and maintained the tubes in a boiling water bath for 1–2 h or until no unsolubilized material remained. Each sample was then diluted with ultrapure H_2O to a weight of 10 g and analyzed for Pb with a Perkin-Elmer model 2200 AAS equipped with a heated graphite atomizer and a model AS-1 auto sampling system, and for Zn with a Perkin-Elmer model 603 AAS. The culture tubes were then emptied, rinsed with ultrapure H_2O, dried in an oven at 80°C, and weighed again. A wet to dry weight ratio (WDR) was computed for each sample. Concentrations of Pb and Zn were reported as micrograms per gram dry weight with a detection limit of 0.01 μg·g^{-1}. Samples of reference material (NBS bovine liver, 0.34 ± 0.08 μg Pb·g^{-1}) carried through this procedure averaged 0.34 μg Pb·g^{-1} (SD = 0.026).

The Statistical Analysis System (SAS Institute 1982a, 1982b), available at the University of Missouri-Columbia, was used for all data handling and statistical analyses. We reduced the heterogeneity of the sample variances by applying the $\log_{10}(x + 1)$ transformation before computing "geometric" means for each collection site. One-way analysis of variance (ANOVA) and Fischer's protected LSD (Chew 1977) were used to compare and test differences among the location means. Relations between variables were investigated more thoroughly with simple correlation analysis, geometric mean regression (Ricker 1973), and stepwise multiple regression (Draper and Smith 1981). We used a probability level of $P \leq 0.05$ in all tests of hypotheses.

Results

The northern hogsuckers were smaller than most of the black and golden redhorse (Table 1). The means for both TL and weight differed significantly among locations, with the differences in weight being especially pronounced. The mean weight of fish in the sample from Leadwood, which contained only northern hogsuckers, was less than any of the other sites. However, none of the location means for the blood variables, WDR, Hb, DNA, and protein, were significantly different (Table 1), indicating that location-wise comparisons of blood-Pb, blood-Zn, and ALA-D activity standardized to any of these variables were not confounded with species effects. The unweighted grand means for the blood variables were WDR, 8.69; Hb, 4.61 mg·mL^{-1}; DNA, 6.72 mg·mL^{-1}; and protein, 72.9 mg·mL^{-1}. Among these variables, the coefficient of variation (CV) was lowest for protein and highest for DNA (Table 1).

Lead concentrations in edible tissue essentially confirmed the earlier findings of the Missouri Department of Conservation. At Desloge, the most contaminated site, concentrations ranged from 0.35 to 1.30 μg·g^{-1}; at Irondale, the site unaffected by tailings, the highest concentration was only 0.13 μg·g^{-1}. Differences among the mean concentrations for each site were significant (Table 1). Lead levels in the edible tissues of fish from Leadwood and Washington State Park were intermediate relative to those from Irondale and Desloge and were not significantly different from each other (Table 1). Concentrations of Pb in the edible tissues of all 10 specimens from Desloge and in 11 of the 15 total specimens from Leadwood and Washington State Park exceeded the 0.3 μg·g^{-1} guideline for human consumption (WHO 1972); none of the specimens from Irondale exceeded 0.3 μg·g^{-1} (Fig. 2).

Concentrations of Pb and Zn in blood essentially paralleled those in edible tissue. Blood-Pb and blood-Zn levels were generally highest at Desloge, intermediate at Leadwood and Washington State Park, and lowest at Irondale (Table 1). For blood-Pb, the location effect was always significant, regardless of whether the concentrations were expressed in terms of blood

TABLE 1. Geometric means for whole fish and blood variables for each site with tests of significance (as F-values) and coefficients of variation (CV). Means followed by the same letter are not significantly different ($P > 0.05$, Fischer's protected LSD).

Variable	Site[a]					
	Irondale[b]	Leadwood[c]	Desloge[d]	Washington State Park[e]	F[f]	CV (%)
Whole fish						
TL (cm)	25.4 a	21.3 a	28.4 a	27.7 a	2.11	6.6
Weight (g)	227.6 a	98.0 b	274.1 a	254.1 a	6.89**	8.0
Pb in edible tissues (wet wt, $\mu g \cdot g^{-1}$)	0.07 a	0.44 b	0.79 c	0.38 b	20.66**	43.8
Blood						
WDR ($g \cdot g^{-1}$)	8.92 a	9.07 a	8.83 a	7.96 a	0.56	22.9
Hb ($mg \cdot mL^{-1}$)	4.13 a	5.34 a	4.54 a	4.50 a	1.08	12.6
DNA ($mg \cdot mL^{-1}$)	6.61 a	7.75 a	6.99 a	5.67 a	0.42	23.7
Protein ($mg \cdot mL^{-1}$)	71.03 a	71.89 a	72.10 a	76.72 a	0.38	4.0
ALA-D ($nmol \cdot L^{-1}$ PBG$\cdot$mL-h^{-1})	222.92 a	139.89 ab	81.76 c	114.09 bc	8.28**	9.4
ALA-D$\cdot Hb^{-1}$ ($nmol \cdot L^{-1}$ PBG$\cdot mg\text{-}h^{-1}$)	54.52 a	26.26 b	18.10 b	25.65 b	15.99**	10.4
ALA-D$\cdot DNA^{-1}$ ($nmol \cdot L^{-1}$ PBG$\cdot mg\text{-}h^{-1}$)	35.15 a	18.11 b	12.46 b	21.91 ab	3.19*	23.5
ALA-D$\cdot protein^{-1}$($nmol \cdot L^{-1}$ PBG$\cdot mg\text{-}h^{-1}$)	3.19 a	1.95 b	1.19 c	1.53 bc	13.30**	22.9
Pb (dry, $\mu g \cdot g^{-1}$)	0.64 a	5.19 b	14.88 c	4.32 b	34.72**	29.9
Pb (wet, $mg \cdot L^{-1}$)	0.08 a	0.80 b	1.81 c	0.63 b	24.94**	46.2
Pb$\cdot Hb^{-1}$ ($\mu g \cdot mg^{-1}$)	0.02 a	0.14 b	0.41 c	0.14 b	26.61**	51.0
Pb$\cdot DNA^{-1}$ ($\mu g \cdot mg^{-1}$)	0.03 a	0.10 ab	0.28 c	0.14 b	8.38**	76.4
Pb$\cdot protein^{-1}$ ($\mu g \cdot mg^{-1}$)	0.001 a	0.011 b	0.027 c	0.009 ab	16.62**	70.1
Zn (dry, $\mu g \cdot g^{-1}$)	75.86 a	94.14 ab	118.72 b	86.42 a	4.54*	6.1
Zn (wet, $mg \cdot L^{-1}$)	8.69 a	10.84 ab	13.37 c	11.18 bc	5.15*	9.7
Zn$\cdot Hb^{-1}$ ($\mu g \cdot mg^{-1}$)	2.17 a	1.94 a	3.05 a	2.45 a	2.41	19.5
Zn$\cdot DNA^{-1}$ ($\mu g \cdot mg^{-1}$)	1.47 a	1.31 a	2.18 a	2.15 a	0.88	45.5
Zn$\cdot protein^{-1}$ ($\mu g \cdot mg^{-1}$)	0.13 a	0.14 a	0.20 b	0.15 ab	4.39*	26.7

[a]See Fig. 1 for locations.
[b]$n = 10$ (6 black redhorse, 3 golden redhorse, 1 northern hogsucker).
[c]$n = 5$ (northern hogsuckers).
[d]$n = 10$ (9 golden redhorse, 1 northern hogsucker).
[e]$n = 10$ (3 black redhorse, 3 golden redhorse, 1 northern hogsucker).
[f]*$P \leq 0.05$; **$P \leq 0.01$.

volume, on a dry weight basis, or per unit of Hb, DNA, or protein (Table 1). All of the blood-Pb means for Desloge were significantly greater than the means for the other sites, and all of the blood-Pb means for Irondale were significantly lower than the means for at least two of the other three sites (Table 1). Conversely, none of the mean blood-Pb concentrations in fish from Leadwood and Washington State Park were significantly different from each other. As judged by separation of the blood-Pb means and their respective F-values and CV's (Table 1), the wet- and dry-weight Pb concentrations and Pb$\cdot Hb^{-1}$ were more precise than either Pb$\cdot DNA^{-1}$ or Pb$\cdot protein^{-1}$ and were therefore better indicators of blood-Pb levels.

Blood-Zn differences among the four locations were less pronounced than those for blood-Pb. The location effects for Zn$\cdot Hb^{-1}$ and Zn$\cdot DNA^{-1}$ were not significant (Table 1). Although the location effects for the other measurements of blood-Zn were significant and the mean concentrations were always highest at Desloge and lowest at Irondale, there were few significant differences among means (Table 1). Like blood-Pb, wet- and dry-weight Zn concentrations and Zn$\cdot Hb^{-1}$ had the lowest CV's, indicating that these three measurements were better indicators of blood-Zn levels than was Zn$\cdot DNA^{-1}$. Unlike blood-Pb, however, the F-value for Zn$\cdot protein^{-1}$ was greater than that for Zn$\cdot Hb^{-1}$, despite the higher CV for Zn$\cdot protein^{-1}$ (Table 1).

Location differences for ALA-D activity were always significant, regardless of how we expressed the results (Table 1). ALA-D activity was always highest in the fish from Irondale and lowest in the fish from Desloge, and the means for these sites were always significantly different from each other (Table 1). Like the means for blood-Pb, means for ALA-D activity at Washington State Park and Leadwood were never significantly different from each other, regardless of how we expressed enzyme activity (Table 1). The CV's were lowest for activity expressed in enzyme units and for ALA-D$\cdot Hb^{-1}$ (Table 1). Although all F-values were significant, those for ALA-D$\cdot Hb^{-1}$ and for specific enzymatic activity (ALA-D$\cdot protein^{-1}$) were greater than the F-values for ALA-D$\cdot DNA^{-1}$ and for activity expressed in enzyme units. Trends among ALA-D means for the affected sites (Leadwood, Desloge, and Washington State Park), expressed as percentages of the means for Irondale, were similar to those described for the original data; mean ALA-D activity was 48–63% of the Irondale mean at Leadwood and Washington State Park, and 33–37% at Desloge (Table 2).

Using geometric mean regression (Ricker 1973), we fit the following functional regression model to the results for blood-Pb (x_{1i}) and edible tissue Pb(x_{2i}):

$$\log_{10}(x_{1i} + 1) = u + v \log_{10}(x_{2i} + 1) + \epsilon_i$$

where ϵ_i is random error, u is the intercept, and v is the regression coefficient. For wet-weight blood-Pb, the relation defined by $u = 0.017$ (± 0.041 (95% confidence limit)) and $v =$

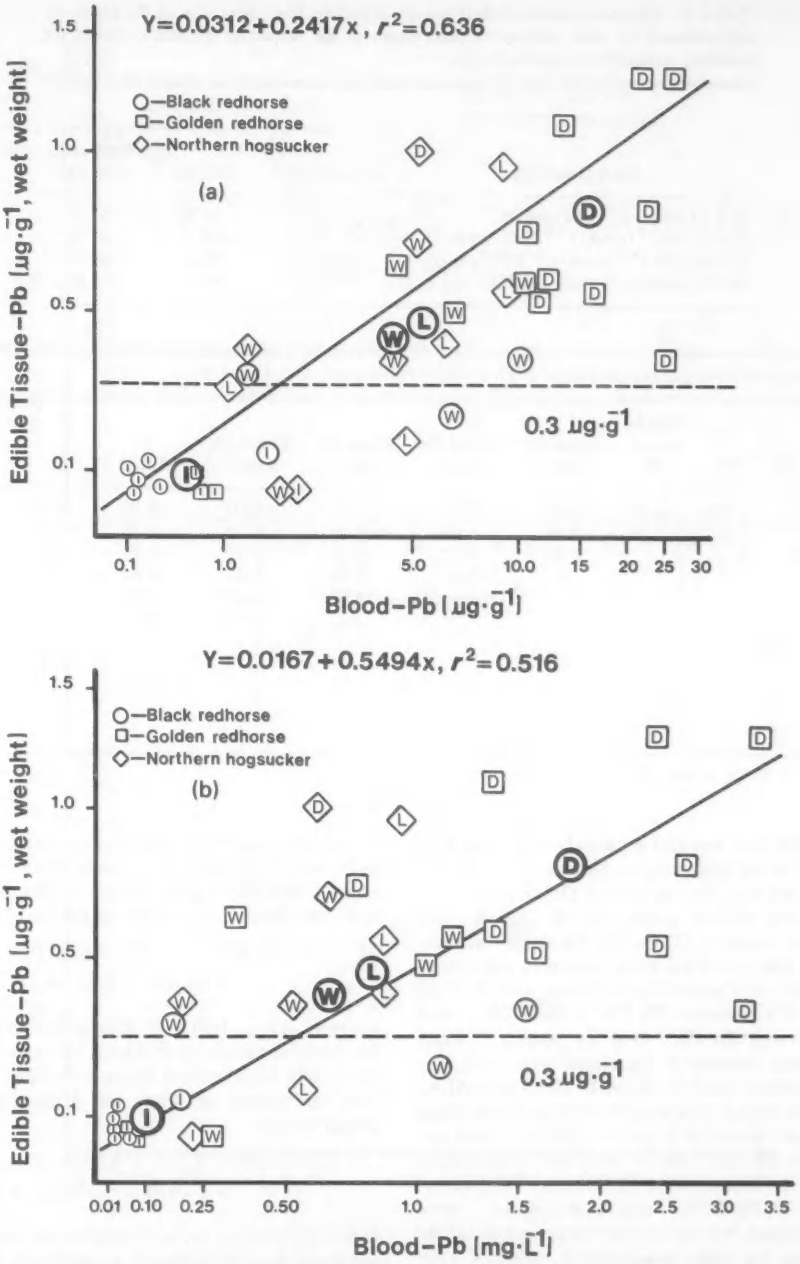

FIG. 2. Log–log, geometric mean regressions between Pb concentrations in edible tissues (*y*-axes) and (a) wet-weight and (b) dry-weight blood-Pb concentrations (*x*-axes). I, Irondale; L, Leadwood; D, Desloge; W, Washington State Park. Values in boldface are geometric means for each site; broken lines represent the current guideline for human consumption (WHO 1972).

(0.550 ± 0.135) accounted for 51.6% of the observed variability (Fig. 2a). For dry-weight blood-Pb, the relation was $u = (0.031 \pm 0.043)$ and $v = (0.242 \pm 0.051)$, which accounted for 63.6% of the observed variability (Fig. 2b). Both regression coefficients, but neither intercept, differed significantly from 0, and both relations were highly significant overall ($P < 0.01$).

The transformed, but unstandardized blood-Pb concentrations (both wet and dry weight) were also positively correlated with blood-Zn levels (Table 3). ALA-D activity expressed in enzyme units was negatively correlated with both unstandardized values for blood-Pb (Fig. 3a, 3b), but only the dry weight correlation was significant for unstandardized blood-Zn vs.

TABLE 2. Geometric mean ALA-D activity in suckers from three sites on the Big River contaminated by mine tailings as percentages of the respective geometric means for Irondale, an unaffected upstream site.

ALA-D activity	Site		
	Leadwood	Desloge	Washington State Park
ALA-D (nmol·L^{-1} PBG·mL-h^{-1})	62.8	36.7	51.2
ALA-D·Hb^{-1} (nmol·L^{-1} PBG·mg-h^{-1})	48.2	33.2	47.0
ALA-D·DNA^{-1} (nmol·L^{-1} PBG·mg-h^{-1})	51.5	35.4	62.3
ALA-D·$protein^{-1}$(nmol·L^{-1} PBG·mg-h^{-1})	61.1	37.3	48.0

TABLE 3. Pearson product-moment correlation coefficients ($n = 35$)[a] between log-transformed concentrations of Hb, DNA, protein, Pb and Zn in blood, fish length and weight, concentrations of Pb in edible tissues, and ALA-D activity.

	TL	Wt	Edible tissue Pb	Blood-Pb (dry)	Blood-Pb (wet)	Blood-Zn (dry)	Blood-Zn (wet)	ALA-D	Hb	DNA	Protein
TL	—	0.76	0.03	0.15	0.13	0.07	−0.03	−0.20	−0.16	0.09	−0.01
Wt		—	−0.09	0.04	0.05	−0.10	−0.12	−0.26	−0.24	0.11	0.13
Edible tissue Pb			—	0.80	0.72	0.61	0.53	−0.53	0.05	−0.10	−0.15
Blood-Pb (dry)				—	0.96	0.58	0.60	−0.64	0.21	0.08	0.04
Blood-Pb (wet)					—	0.50	0.65	−0.56	0.31	0.17	0.08
Blood-Zn (dry)						—	0.79	−0.37	−0.26	−0.03	−0.32
Blood-Zn (wet)							—	−0.17	0.24	0.11	0.14
ALA-D								—	0.33	0.08	0.33
Hb									—	0.40	0.75
DNA										—	0.32
Protein											—

[a] $|r| \geq 0.33$, $P \leq 0.05$; $|r| \geq 0.42$, $P \leq 0.01$.

ALA-D (Table 3). ALA-D was also negatively correlated with Pb in edible tissue. The only other unstandardized variables that were highly correlated were Hb and protein (Table 3).

Similar trends were evident among the Hb-, DNA-, and protein-standardized variables (Table 4). All three measurements of blood-Pb and blood-Zn were positively correlated. Lead in edible tissues was positively correlated with all three standardized blood-Pb variables (Pb·Hb^{-1}, Pb·DNA^{-1}, and Pb·$protein^{-1}$) and with Zn·Hb^{-1} and Zn·$protein^{-1}$. Lead in edible tissues was negatively correlated with both Hb- and protein-standardized ALA-D (ALA-D·Hb^{-1} and ALA-D·$protein^{-1}$), but the negative correlation between edible tissue Pb and DNA-standardized ALA-D (ALA-D·DNA^{-1}) was not significant (Table 4). Pb·Hb^{-1} and ALA-D·Hb^{-1} were negatively correlated (Fig. 3b), as were Pb·$protein^{-1}$ and ALA-D·$protein^{-1}$ (Table 4). Pb·DNA^{-1} and ALA-D·DNA^{-1} were also negatively correlated, but neither this relation nor any of the correlations between the other standardized variables were statistically significant (Table 4).

We used the forward selection version of multiple linear regression (SAS Institute 1982b) to quantify the relations between ALA-D activity and blood-Pb and blood-Zn concentrations, edible tissue Pb residues, and the blood variables. Our criterion for model selection was that a variable would be added only if it significantly ($P \leq 0.05$) reduced the unexplained sum-of-squares. We first fit a model in which ALA-D in enzyme units ($\hat{Y}_i$) was regressed against linear, quadratic, and interaction variables representing unstandardized dry-weight concentrations of Pb (x_{1i}) and Zn (x_{2i}) in blood (micrograms per gram), Hb, DNA, and protein concentrations (milligrams per millilitre), WDR, and edible tissue Pb (micrograms per gram), with all variables log-transformed. The model selected, with 95% confidence limits on the regression coefficients, was

$$\hat{Y}_i = -0.0661 - (0.597 \pm 0.153)x_{1i} + (0.686 \pm 0.483)x_{2i} + (1.715 \pm 0.591)x_{3i}$$

where x_{3i} is $\log_{10}(\text{Hb} + 1)$. This model accounted for 73.5% of the observed variability in ALA-D activity. Replacement of the dry-weight blood metals terms with their wet-weight equivalents (milligrams per litre) and deletion of WDR yielded a similar model:

$$\hat{Y}_i = 0.608 - (2.711 \pm 0.868)x_{1i} + (2.523 \pm 1.369)x_{1i}^2 + (0.676 \pm 0.506)x_{2i} + (1.598 \pm 0.525)x_{3i}$$

where x_{1i} and x_{2i} are wet-weight, log-transformed blood-Pb and blood-Zn concentrations, respectively, and $\hat{Y}_i$ and x_{3i} are as before. This model accounted for 75.8% of the observed variability in ALA-D activity.

We next regressed log-transformed ALA-D·Hb^{-1}, ALA-D·DNA^{-1}, and ALA-D·$protein^{-1}$ ($\hat{Y}_i$) against log-transformed, standardized variables representing the blood metals (x_{1i} = blood-Pb and x_{2i} = blood-Zn) (micrograms per milligram) and edible tissue Pb (micrograms per gram). With all blood variables standardized to Hb, the model selected was

$$\hat{Y}_i = 1.588 - (8.340 \pm 2.179)x_{1i} + (27.232 \pm 10.138)x_{1i}^2 + (0.425 \pm 0.414)x_{2i}$$

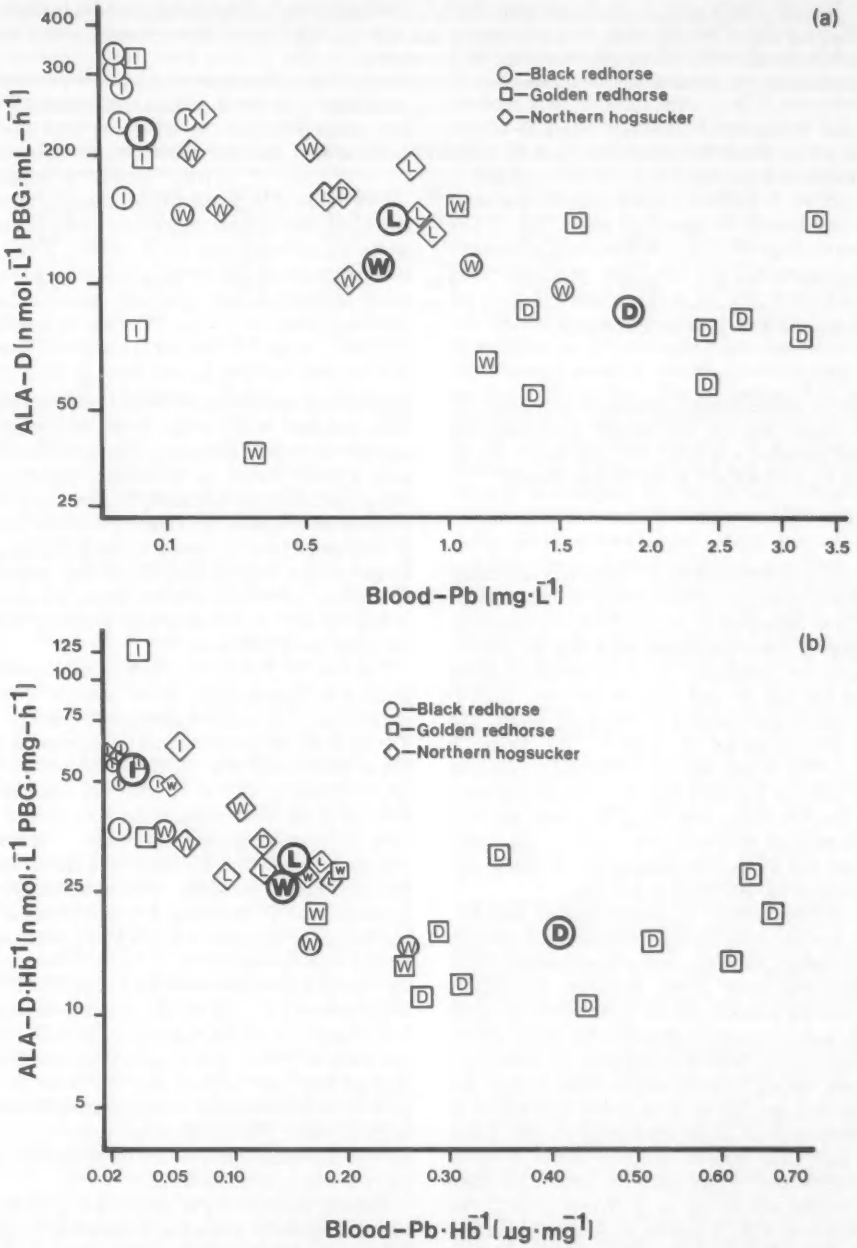

FIG. 3. Log–log plots of blood-Pb concentrations (*x*-axes) and ALA-D activity (*y*-axes) based on (a) unstandardized measurements and (b) with both variables standardized to Hb concentrations. I, Irondale; L, Leadwood; D, Desloge; W, Washington State Park. Values in boldface are geometric means for each site.

which accounted for 75.6% of the observed variability in ALA-D·Hb^{-1}. Multiple regression with DNA- and protein-standardized variables accounted for less variability, 68.5 and 56.4% for ALA-D·DNA^{-1} and ALA-D·$protein^{-1}$, respectively. Nevertheless, all five of the multiple regression equations were highly significant ($P < 0.01$), all accounted for more than 50% of the observed variability in ALA-D activity, and a variable representing blood-Pb was always selected first, with a negative regression coefficient. Additionally, all the models except the one based on protein-standardized variables included a positive regression coefficient for at least one variable representing blood-Zn, and none included terms representing

TABLE 4. Pearson product-moment correlation coefficients ($n = 35$)[a] between log-transformed blood concentrations of Pb and Zn, fish length and weight, Pb concentrations in edible tissues, and ALA-D activity. (A) All blood variables, per unit Hb; (B) all blood variables, per unit DNA; (C) all blood variables, per unit protein.

	(A) Hb^{-1}			(B) DNA^{-1}			(C) $Protein^{-1}$		
	Blood-Pb	Blood-Zn	ALA-D	Blood-Pb	Blood-Zn	ALA-D	Blood-Pb	Blood-Zn	ALA-D
TL	0.17	0.11	−0.12	0.03	−0.09	−0.20	0.10	0.01	−0.16
Wt	0.11	0.10	−0.15	−0.06	−0.16	−0.27	0.03	−0.12	−0.26
Blood-Pb	—	0.39	−0.70	—	0.70	0.10	—	0.61	−0.50
Blood-Zn	—	—	−0.13	—	—	0.62	—	—	−0.25
Edible tissue Pb	0.73	0.40	−0.59	0.68	0.31	−0.30	0.69	0.59	−0.53

[a] $|r| \geq 0.33$, $P \leq 0.05$; $|r| \geq 0.42$, $P \leq 0.01$.

Pb concentrations in edible tissues. Based on r^2 values, the Hb-standardized model and the wet-weight, unstandardized model, which also included a term for Hb, appeared to be the best estimators of ALA-D activity in the 35-fish data set.

Discussion

The measurement of erythrocyte ALA-D activity can be used to document exposure of fish to environmental Pb. In our study, enzyme activity was depressed at all three sites downstream from known sources of Pb-contaminated mine tailings, and we were able to describe significant relations between blood concentrations of Pb and Zn and enzyme activity. ALA-D activity was negatively correlated with blood-Pb levels, and Hb-standardized concentrations of blood-Pb and blood-Zn accounted for over 75% of the observed variability in enzyme activity. ALA-D activity was also negatively correlated with edible tissue Pb concentrations, and the edible tissue residues were positively correlated with blood-Pb. Collectively, these results demonstrate that Pb is both biologically available and biochemically active in the Big River ecosystem.

The analysis of Pb residues in environmental samples, especially in fish, is notoriously difficult because contamination during collection, storage, handling, and preparation is difficult to avoid (Patterson and Settle 1976), and there is typically much variability among samples caused by the heterogeneous distribution of Pb within organisms (Phillips and Russo 1978). Results are therefore always difficult to interpret, and even more difficult to compare among different studies. The ALA-D and blood metals approach appears to be a usable supplement to tissue residue determinations in environmental studies. Blood samples are less likely than samples of whole fish or of edible tissues to be contaminated before analysis, especially when collected with a needle and syringe (e.g. Wiener 1982). The findings by Hodson et al. (1977) that the ALA-D response was insensitive to Pb added to the blood sample should further lessen the risk of contamination. Because the inactivation of ALA-D is a biochemical response that is specific for Pb, the results should also be easier to interpret than tissue concentrations alone.

Although our results indicate an acceptable level of precision for most of the variables we measured, there are two immediately obvious areas for improvement. First, our study was conducted during spring, when catostomids typically move upstream to spawn (e.g. Scott and Crossman 1973). The possible movement of the fish prior to their capture and the rapid changes in streamflow typical of spring in Missouri would vary the concentrations of Pb and Zn to which the fish had been exposed and may have contributed to within-location variation. Late summer, when water levels are relatively stable and suckers are more restricted in their movements, would have been a better season for collection. Second, the necessity of using more than one species in our "experiment" may also have contributed to within-site variability. However, the inclusion of several species did not appear to confound our study design; the blood variables were similar for all three species (Table 1), and none of the points that represent northern hogsuckers and golden redhorse in Fig. 3a and 3b deviate markedly from the points that represent black redhorse.

The use of Hb to standardize measurements of ALA-D activity in homogenized blood samples offers an expedient alternative to the more traditional hematocrit or RBC counts. The assay for Hb proved easier to perform on small volumes of blood than either the DNA or the protein assay and was relatively precise (CV = 12.6%). The DNA assay was the most difficult of the three and was the least precise (CV = 23.7%). Both of the multiple regression models selected to describe the relation between ALA-D activity and blood metals incorporated Hb-standardized variables. With unstandardized data, Hb was accounted for by including it in the model as an independent variable; expressing ALA-D and blood metals on a unit Hb basis proved equally successful. Because differences in Hb among the four locations were not significant (Table 2), standardization of the results to Hb did not diminish the measured effects of the blood metals on ALA-D activity. However, Johansson-Sjöbeck and Larsson (1979) and Krajnovic-Ozretic and Ozretic (1980) showed that both ALA-D and Hb could be reduced in fish exposed in the laboratory to high concentrations (>300 $\mu g \cdot L^{-1}$) of Pb in water. We therefore recognize that at blood-Pb levels sufficient to measurably lessen Hb synthesis, diminution of the ALA-D response could occur.

Another shortcoming of standardizing ALA-D activity to Hb becomes apparent when one attempts to compare results with values reported in the literature, where enzyme activity has traditionally been reported as ALA-D·mL RBC^{-1}. Although we have no hematocrit or RBC data for our three sucker species, most of our mean Hb values (Table 1) were lower than the 5–11 $mg \cdot mL^{-1}$ range reported by Lockhart and Lutz (1977) for white suckers (*Catostomus commersoni*). However, they fall within the range listed by Satchell (1971) for other teleosts. Lead in blood is almost completely associated with erythrocytes (Rosen and Sorell 1978; Posner et al. 1978), as are ALA-D and Hb. Conversely, DNA and protein are present in all types of blood cells, and serum protein levels of 4–6 $mg \cdot mL^{-1}$ have been measured in catostomids (Lockhart and Lutz 1977).

Houston and DeWilde (1968) found that Hb in rainbow trout blood could be estimated reasonably well by using RBC counts, microhematocrit measurements, and packed cell volumes. Because these erythrocyte variables are closely relaed, values for ALA-D activity from several studies, where the results have been expressed in different units of standardization, can probably be compared when all the data are converted to percentages of control or "normal" activity.

Exposure concentrations in the Big River were within the 0–100 $\mu g\ Pb \cdot L^{-1}$ range used in laboratory studies with salmonids by Hodson et al. (1977, 1978). Total dissolved Pb (that which passes through a 0.45-μm filter) in the tailings-contaminated reaches varied with stream discharge over a range of about 10–20 $\mu g \cdot L^{-1}$ at Desloge and 5–20 $\mu g \cdot L^{-1}$ at Washington State Park; concentrations at Irondale never exceed 5 $\mu g \cdot L^{-1}$ (Schmitt and Finger 1982). Typical conditions (at median flow) were as follows: Desloge — pH 7.7, hardness 170 $mg \cdot L^{-1}$, alkalinity 133 $mg \cdot L^{-1}$, and total dissolved Pb about 11 $\mu g \cdot L^{-1}$; and Washington State Park — pH 7.8, hardness 225 $mg \cdot L^{-1}$, alkalinity 165 $mg \cdot L^{-1}$, and total dissolved Pb about 7 $\mu g \cdot L^{-1}$. Hodson et al. (1977, 1978) maintained somewhat softer, less alkaline water conditions (pH 7.7, hardness 135 $mg \cdot L^{-1}$, alkalinity 80 $mg \cdot L^{-1}$) in their laboratory studies. Our calculations based on the REDEQL·EPAK model (Ingle et al. 1980) suggest that at 13 μg total $Pb \cdot L^{-1}$, the distribution of dissolved Pb species at equilibrium is similar under either set of conditions; virtually all liquid-phase Pb occurs as carbonate complexes, with little free Pb^{2+} present (3.5% free ion in the Big River, 7.5% in Lake Ontario). Although we can compare our results with those reported by Hodson et al. (1977, 1978), we cannot discount entirely the contribution of dietary Pb in our study; the aquatic flora and fauna of the Big River contain relatively high Pb, Zn, and Cd levels (Schmitt and Finger 1982). Some studies have shown the dietary route to be important in the uptake of Pb by fish (e.g. Vighi 1981); however, Hodson et al. (1978) have shown it to be unimportant relative to uptake from the water.

ALA-D activity (as $ALA\text{-}D \cdot Hb^{-1}$) in our study was depressed by about 50% at Washington State Park, where dissolved Pb averaged 7 $\mu g \cdot L^{-1}$, and by 67% at Desloge, where dissolved Pb averaged 12 $\mu g \cdot L^{-1}$. The ALA-D response to waterborne Pb therefore appears as sensitive in the three catostomids collected from the Big River as it is in rainbow trout, for which Hodson et al. (1977, 1978) reported reduced enzyme activity at their lowest nominal treatment level of 10 $\mu g \cdot L^{-1}$ (about 13 $\mu g \cdot L^{-1}$ measured). These concentrations are all less than the 18.2–37.7 $\mu g \cdot L^{-1}$ maximum acceptable toxicant concentration (MATC) reported by Davies et al. (1976) for rainbow trout exposed to Pb in hard water. Concentrations of Pb at the affected Big River sites were within or slightly higher than the 4.1–7.6 $\mu g \cdot L^{-1}$ MATC range reported for rainbow trout that had been exposed to Pb in soft water when exposure began at the eyed egg stage (Davies et al. 1976). Collectively, these findings indicate that ALA-D is more sensitive to Pb than are traditional toxicity tests.

In general, there is no known threshold blood-Pb concentration that must be achieved to inhibit ALA-D. In humans, the ALA-D activity response is essentially linear over the range of 100–900 $\mu g \cdot L^{-1}$ (Rosen and Sorell 1978); complete inhibition occurs near the upper end of this range. Dieter and Finley (1978) showed a linear response over a blood-Pb range of about 50–500 $\mu g \cdot L^{-1}$ in mallards (*Anas platyrhynchos*) that had been given a proventricular dose of lead shot, and Dieter (1979) reported similar results over a range of about 30–1000 $\mu g \cdot L^{-1}$ in wild canvasbacks (*Aythya valisineria*) that had ingested lead shot. Hodson et al. (1977, 1978) showed linear responses in rainbow trout with blood-Pb levels of 30–5400 $\mu g \cdot L^{-1}$. Although brook trout (*Salvelinus fontinalis*) accumulated less Pb and consequently appeared less sensitive to exposure than rainbow trout, Hodson et al. (1977) nevertheless felt that the enzyme response would have been linear had a wider range of blood-Pb and enzyme activity values been available.

The blood-Pb levels we measured in suckers from contaminated and uncontaminated reaches of the Big River (Tables 1 and 2) fell within the range of control and treatment levels reported by Hodson et al. (1978) for rainbow trout exposed to Pb in the laboratory. However, our data suggest a curvilinearity in the ALA-D response (Fig. 3a, 3b); there was proportionately less decline in enzyme activity at high than at low blood-Pb concentrations. Our multiple regression results suggest that this curvilinearity resulted from the co-occurrence of Zn and Pb in the blood of our fish. The activation of ALA-D requires the presence of Zn, and its antagonistic effect on ALA-D inhibition by Pb has been demonstrated in mammals (Finelli 1977). Hodson et al. (1977) reported that some workers have shown ALA-D stimulation by Zn in fish. Krajnovic-Ozretic and Ozretic (1980) showed that mixtures of Pb and Zn added in vitro to the blood of gray mullet (*Mugil auratus*) could lessen the negative effects of previous exposure to Pb on ALA-D activity. Hutton (1983) demonstrated a similar response to Zn in the blood of feral pigeons (*Columba livia*) from urban areas. Erythrocyte-Pb can be displaced by Zn as well as by certain other divalent cations (Rosen and Sorell 1978; Finelli 1977). Dissolved Zn levels average 60–100 $\mu g \cdot L^{-1}$ in tailings-contaminated reaches of the Big River (Schmitt and Finger 1982). Although these concentrations are below the levels considered harmful to fish in hard water (e.g. Brungs 1969), the presence of significantly higher blood-Zn residues in suckers from tailings-contaminated sites (Table 2) illustrates the availability of Zn to the biota. Our data suggest that blood-Zn mediates the inhibition of ALA-D by blood-Pb in fish exposed to environmental Pb as it does in mammals and in the blood of fish and birds to which Zn has been added in vitro. However, we also recognize the obvious colinearity between Pb and Zn concentrations in all of our data. A factorially arranged laboratory experiment would be required to fully differentiate the two effects and their interations, and to attempt elucidation of the mechanism involved.

Because ALA-D is apparently present in excess (Posner et al. 1978), low-level inhibition of this enzyme is not generally considered a toxic response. Rather, it is an indication that the organism has recently been exposed to Pb. Chronic exposure of fish to environmental Pb should ultimately result in black tail, lordoscoliosis, and behavioral abnormalities (e.g. Hodson et al. 1978). We have seen no evidence of such symptoms in the several hundred fish from the Big River that we have handled, only elevated metals concentrations and reduced ALA-D activity in suckers. We have, however, demonstrated that the modified ALA-D assay and measurements of Hb, Pb, and Zn in fish blood can be used to document the bioavailability and biochemical activity of Pb at sublethal levels in the aquatic environment.

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Restriction Endonuclease Analysis of Salmonid Mitochondrial DNA

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We have analyzed the mitochondrial DNA from four species of the teleost subfamily Salmoninae using restriction endonucleases and found that the salmonid mitochondrial genome is slightly larger than that of the human, about 16 670 base pairs. Systematic analyses by both cladistic and phenetic methods suggest a closer phylogenetic relationship between *Oncorhynchus tshawytscha* and *Salmo (Parasalmo) gairdneri* than either has with *Salmo (Salmo) trutta*. *Salvelinus fontinalis* appears to be most distantly related of these four species. Applications of this molecular technique to a variety of genetic and population studies appear promising.

Nous avons analysé l'ADN des mitochondries de quatre espèces de poissons téléostéens de la sous-famille des Salmoninæ en utilisant des endonucléases restrictives. Les résulates montrent que le génome des salmonidés est légèrement plus large que celui de l'homme car il contient près de 16 670 paires de bases. Des analyses systématiques ont été faites par les méthodes cladistiques et phénétiques. Les résultats suggèrent une plus grande affinité phyogénétique entre *Oncorhynchus tshawytscha* et *Salmo (Parasalmo) gairdneri* que celle existente entre ceux deux espèces et *Salmo (Salmo) trutta*. *Salvelinus fontinalis* semble avoir la plus grande divergence génétique dans l'ensemble des quatre espèces. Ce type d'études semble indiquer importantes applications de cette technique moléculaire pour an nombre d'études génétiques et population.

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The teleost subfamily Salmoninae consists of 25–35 species provisionally partitioned into two tribes, Salmonini and Salvelini (Behnke 1965, 1968). These species are of commercial and scientific interest and many studies have been done to clarify their systematic relationships. Data from studies on morphology, ecology, and biogeography (Rounsefell 1962; Valdykov 1963; Behnke 1965, 1972), paleontology (Cavender and Miller 1972; Taylor and Smith 1981), karyology (Simon 1963; Gold et al. 1977; Gorshkov and Gorshkova 1981), physiology (Hoar 1976), embryology (Pavlov 1980), and nuclear DNA hybridization (Mednikov and Akhundov 1975; Gharrett et al. 1977; Hanham and Smith 1980; Schmidtke and Kandt 1981) are available and support the broad outlines of generic relationships described by Behnke (1968) yet leave unresolved many questions of species level systematics. Use of starch gel electrophoresis has provided a finer grain view of inter- and intra-specific relationships (Utter et al. 1973; Loudenslager and Gall 1980; Milner et al. 1981) but is not without limitations (Allendorf and Utter 1979; Utter 1981), some of which stem from the tetraploid origin of salmonids (Allendorf et al. 1975). This paper reports the application of a new molecular technique to examine systematic relationships within Salmoninae at three taxonomic levels: tribe, genus, and subgenus.

Restriction endonucleases cleave DNA, producing fragments, at specific recognition sites which consist of short, palindromic sequences of four, five, or six nucleotides (Boyer 1971, 1974). Animal mitochondrial DNA (mtDNA) is a covalently closed circular DNA duplex (Brown and Vinograd 1974; Parker et al. 1977) that is maternally inherited (Giles et al. 1980), genetically autonomous (Birky 1978), occurs in about 1000 copies per cell (Bogenhagen and Clayton 1974), and evolves 5–10 times more rapidly than single copy nuclear DNA (Brown et al. 1979, 1982).

Mitochondrial DNA lacks many of the complicating features of nuclear DNA (Schmidtke and Epplen 1980; Brown 1981) allowing direct analysis of the genotype; thus, comparative analysis of mtDNA should yield detailed information concerning relationships of closely related species or populations. Comparison of fragment patterns produced by digestion of mtDNA molecules by restriction endonucleases has already proved useful in systematic and population studies of a variety of animal groups (Upholt and Dawid 1977; Avise et al. 1979a, 1979b; Brown and Wright 1979; Ferris et al. 1981b, 1982; Shah and Langley 1979; Yonekawa et al. 1981).

Our objective was to apply this method in teleost fish systematics. In particular, we used restriction endonucleases (EC 3.1.23) to analyze mtDNA from four salmonid species: *Salvelinus fontinalis* (brook trout), *Oncorhynchus tshawytscha* (chinook salmon), *Salmo (Salmo) trutta* (brown trout), and *Salmo (Parasalmo) gairdneri* (rainbow trout).

Materials and Methods

Six rainbow trout, known to be at least maternal half-sibs, were obtained from the University of California, Davis hatchery

stock called RTD. One brown trout and one brook trout were acquired from the California Department of Fish and Game hatcheries at Mt. Shasta, CA, and San Joaquin, CA, respectively. Two chinook salmon were caught in the open sea 16 km off the coast of San Francisco, CA.

Mitochondrial DNA was purified from livers of freshly sacrificed animals according to the procedure of Brown et al. (1979). Tissues from two sets of three rainbow trout each were combined to obtain sufficient material. The only modification of the Brown et al. (1979) cesium chloride ultracentrifugation procedure was in collection of DNA after the first equilibrium gradient. Because only a wide nuclear DNA band was evident, the bottom 8–10 drops of each band were collected and rebanded in cesium chloride. Diluting the total DNA (thus enriching for mtDNA) resulted in a visible mtDNA band upon recentrifugation.

About 20 ng of mtDNA was digested with restriction endonucleases according to supplier's directions (New England Biolabs). Fragments were labelled at the ends with ^{32}P according to the procedure of Brown (1980) and separated electophoretically in 1.2% agarose or 3.5% acrylamide gels. Autoradiography was done overnight with a Dupont Cronex screen. Sizes of fragments were estimated by algebraic and graphical methods using PM2 and lamda phage DNA digested with *Hin*dIII as size standards (Southern 1979).

To estimate phylogenetic relationships among the four species, fragments of coincident mobility were initially assumed to be products of single nucleotide substitutions within a restriction site and were considered to be homologous. Fragments were treated as characters, each fragment defined by flanking restriction sites. Character states were coded as either the presence or absence of the fragment. This resulted in a data set of 272 characters, 16 of which were present in all samples and thus had zero phylogenetic information. The remaining 256 characters, consisting of 198 unique characters or evolutionary novelties and 58 phylogenetically informative characters (see Ferris et al. 1981b), were used to test each of the four possible networks obtained for four taxa (Felsenstein 1978). Total length of each network was determined by summing the character state changes among the four species for each character. The network that had the lowest total branch length represents a Wagner network and, by the parsimony criteria, would be accepted as the most reasonable phylogenetic hypothesis.

Ideally, the branch length along a network separating each pair of species would equal the number of character state differences between their data sets. This would result in a lower bound of 256 for the total ideal network length. Total network lengths greater than this lower bound would occur if certain fragments of coincident mobility were not homologous but displayed character state similarities due to parallelism, convergence, or character state reversal. Multiple nucleotide substitutions within a restriction site could result in such homoplastic similarities that would have been initially misinterpreted as homologous states. Since the precise nature and timing of the postulated multiple nucleotide substitutions cannot be made, removal of the nonhomologous fragments of coincident mobility allows a more accurate estimate of the network branch lengths.

The Wagner network was rooted by considering brook trout, a member of the tribe Salvelini, as an out-group taxon for comparison to the remaining three species, all members of the tribe Salmonini. Use of the algorithm of Farris (1970) allows assignment of character state values to the hypothetical taxonomic units (HTUs) linking the four species. Character state values for a hypothetical common ancestor to all four species would be median to those of brook trout and the HTU linking brook trout to the rest of the network. The position of the hypothetical common ancestor would be on the branch linking brook trout to the network at a distance from brook trout equal to the number of character state differences between them.

Percent sequence divergence estimates were calculated using the comparative fragment method (Nei and Li 1979; Nei and Tajima 1981). This method assumes homology of fragments of coincident mobility; thus, fragments sharing homoplastic similarities were not included in the analyzed data set. Sequence divergence estimates tend to be smaller for data obtained from six base enzymes than for data from four base enzymes (George 1982; Tajima and Nei 1982); we therefore pooled the estimates of six and four base enzymes. The unweighted pair-group method using arithmetic averages (UPGMA, Sneath and Sokal 1973) was used to cluster the sequence divergence estimates, producing a phenogram.

Results

Size estimates for the salmonid mtDNA fragments obtained by digestion with 13 restriction enzymes (exclusive of *Mbo*I and *Hinf*I) are given in Tables 1 and 2. The enzyme *Sac*II (top of Table 1) produced two fragments of equivalent mobility in all four salmonid species, constituting strong evidence that their mitochondrial genomes are the same size. *Sac*II produces two cuts in the ribosomal genes of human and mouse mtDNA (Anderson et al. 1981; Bibb et al. 1981) and presumably in salmonid mtDNA as well. Both fragments of rainbow trout mtDNA produced by *Sac*II digestion were slightly larger than those of human mtDNA. We estimate the salmonid mtDNA genome to be about 100 base pairs (bp) larger than human mtDNA, or about 16 670 bp. The mean salmonid mtDNA genome size estimated from the sum of those fragments whose size may be accurately estimated (Tables 1 and 2) is 16 062 bp, or about 4% less than estimated from the *Sac*II comparisons alone.

The fragment patterns produced by the six base enzyme *Xba*I (Fig. 1) are consistent for both rainbow trout samples and for both of the chinook salmon samples. Although three fragments are different between the rainbow trout and chinook salmon patterns, there is a high degree of homology. This difference in patterns is consistent with a single site change (base substitution within a site). We hypothesize that the first and third fragments of the rainbow trout pattern may have fused (by site loss) to give the larger fragment of the chinook salmon pattern. As seen in Table 1 for *Xba*I, this corresponds to summing the 5690 bp and 3080 bp fragments of *S. gairdneri* to the size of the 8770 bp fragment observed in *O. tshawytscha*. Brown trout and brook trout patterns, respectively, show fewer shared fragments with the rainbow trout, in that order listed.

*Hin*dIII patterns from the four species are illustrated in Fig. 2. This enzyme, which also recognizes six bases, yields an identical pattern for rainbow trout and chinook salmon, underscoring their close genetic relationship. As with the previous enzyme digestion, brown trout and brook trout, in the last two lanes, show decreasing numbers of shared fragments with rainbow trout and chinook salmon.

Fragment patterns produced by *Mbo*I and *Hinf*I, enzymes that recognize four base sequences, are shown in Fig. 3. Since

TABLE 1. Sizes (bp) of fragments produced by digestion of the mitochondrial DNAs of four species of salmonids with eight restriction endonucleases. *O.t.* = *Oncorhynchus tshawytscha*, *S.g.* = *Salmo gairdneri*, *S.t.* = *Salmo trutta*, and *S.f.* = *Salvelinus fontinalis*.

	O.t.	*S.g.*	*S.t.*	*S.f.*
		*Sac*II		
	14 920	14 920	14 920	14 920
	1 750	1 750	1 750	1 750
Σ	16 670	16 670	16 670	16 670
		*Hin*dIII		
	6 700	6 700	8 660	5 070
	3 400	3 400	3 400	3 770
	2 170	2 170	2 310	2 980
	1 770	1 770	1 770	2 310
	1 230	1 230	250	1 770
	1 150	1 150		440
	250	250		250
Σ	16 670	16 670	16 390	16 590
		*Pst*I		
	14 790[a]	10 790[a]	12 890[a]	13 675[a]
	1 600	4 000	3 500	1 680
	280	1 600	280	575
		280		460
				280
Σ	16 670[a]	16 670[a]	16 670[a]	16 670[a]
		*Sac*I		
	16 670[a]	16 670[a]	16 670[a]	15 110[a]
				650
				610
				300
Σ	16 670[a]	16 670[a]	16 670[a]	16 670[a]
		*Xba*I		
	8 770	5 690	7 280	6 520
	3 150	3 150	3 150	4 340
	2 310	3 080	3 080	3 080
	1 480	2 310	2 210	2 310
	690	1 480		
		690		
Σ	16 400	16 400	15 720	16 250
		*Bgl*I		
	6 400	5 300	4 100	8 070[a]
	4 100	4 100	4 080	5 800
	3 120	4 080	2 800	2 800
	1 190	2 190	2 775	
	880	880	1 260	
Σ	15 690	16 550	15 015	16 670[a]
		*Bgl*II		
	8 460	11 400	15 410	14 170[a]
	3 010	3 010	810	1 190
	2 210	1 130		1 170
	1 130			140
Σ	14 810	15 540	16 220	16 670[a]
		*Bam*HI		
	11 000	11 000	6 000	11 000
	4 800	4 800	5 300	2 410
			4 800	2 400
Σ	15 800	15 800	16 100	15 810

[a]Fragment size was estimated by subtracting sum of other fragments in the pattern from 16 670 bp.

TABLE 2. Sizes (bp) of fragments produced by digestion of the mitochondrial DNAs of four species of salmonids with five restriction endonucleases. *O.t.* = *Oncorhynchus tshawytscha*, *S.g.* = *Salmo gairdneri*, *S.t.* = *Salmo trutta*, and *S.f.* = *Salvelinus fontinalis*.

	O.t.	*S.g.*	*S.t.*	*S.f.*
		*Pvu*II		
	7 400	7 400	12 970[a]	14 570[a]
	4 580	4 580	3 700	1 310
	2 430	2 450		790
	2 300	2 200		
Σ	16 710	16 630	16 670[a]	16 670[a]
		*Hinc*II		
	5 530	5 530	3 430	3 000
	2 590	3 960	2 650	2 850
	2 360	3 430	2 360	2 360
	2 350	2 570	1 930	2 340
	1 120	450	1 760	1 660
	850		1 000	1 000
	780		810	810
			560	560
Σ	15 580	15 970	14 500	14 580
		*Eco*RI		
	4 170	7 560	8 200	8 470[a]
	3 710	3 710	3 710	8 200
	3 700	3 700	3 700	
	3 330	700	300	
	300	300		
Σ	15 210	15 970	15 910	16 670[a]
		*Ava*II		
	5 660	5 660	5 800	4 300
	2 800	2 605	2 800	2 860
	2 605	2 080	2 605	2 605
	2 080	1 770	2 080	2 080
	1 320	1 320	1 420	1 420
	650	1 270	1 320	1 320
	550	650	950	880
	260	550	820	840
		260	650	
			550	
Σ	15 925	16 165	18 995	16 305
		*Fnu*DII		
	3 500	3 000	3 700	3 500
	3 000	2 430	3 500	2 430
	2 430	2 000	2 430	2 400
	2 100	1 970	1 400	1 500
	1 575	1 290	1 070	1 210
	1 070	1 210	1 065	1 070
	1 065	1 070	1 030	1 030
	940	1 065	540	900
	620	940		540
	540	540		
Σ	16 840	16 135	14 735	14 580

[a]Fragment size was estimated by subtracting sum of other fragments in the pattern from 16 670 bp.

the probability of a specific four base sequence occurring is greater than that for a specific six base sequence, more fragments are produced. Each species has a distinct pattern. The two chinook salmon appear identical.

The fragment pattern assignments, designated by lowercase letters, for each of the 15 enzymes used in this study for the four

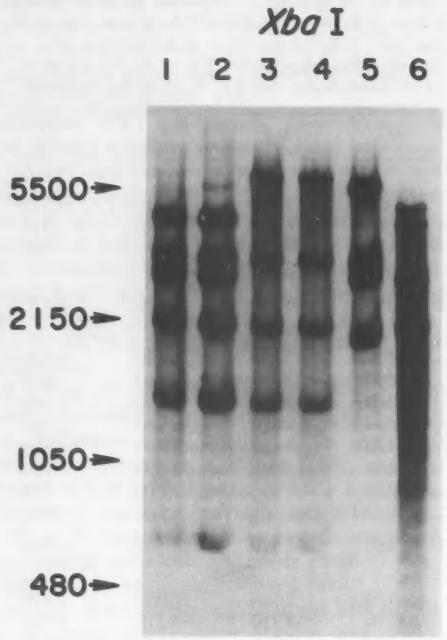

FIG. 1. Mitochondrial DNA fragment patterns produced by digestion with the six base restriction endonuclease *Xba*I. A pattern is defined as the array of fragments produced by digestion with a particular enzyme. Patterns 1 and 2: replicate rainbow trout preparations; 3 and 4: identical chinook salmon; 5: brown trout; 6: brook trout. The numbers at the left are the sizes (bp) of PM2 phage DNA digested with *Hin*dIII.

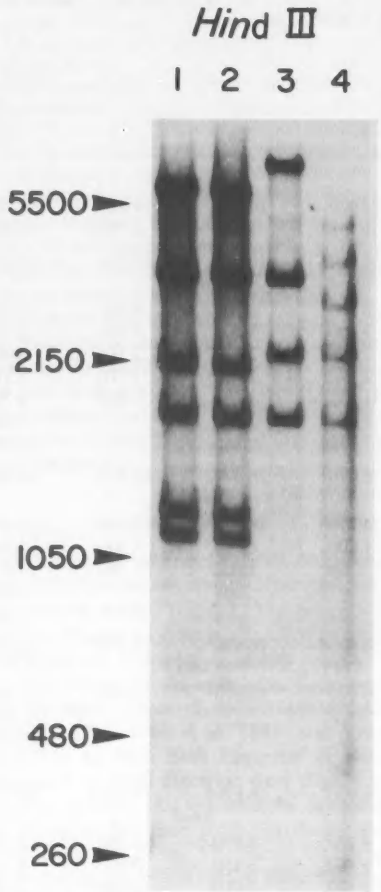

FIG. 2. Mitochondrial DNA fragment patterns produced by digestion with the six base restriction endonuclease *Hin*dIII. Patterns 1 and 2: replicate rainbow trout; 3 and 4: identical chinook salmon; 5: brown trout; 6: brook trout. The numbers at the left are the sizes (bp) of PM2 phage DNA digested with *Hin*dIII.

species are given in Table 3. The close affinities of *S. gairdneri* and *O. tshawytscha* are seen by the sharing of identical patterns for four enzymes, whereas these two species share only two fragment patterns with *S. trutta* and one with *S. fontinalis*.

The network that had the lowest total branch length required explanation of 274 character state changes or 18 presumptive nucleotide substitutions over the lower bound of 256. The other possible networks required at least 287 character state changes and were therefore rejected in favor of the shortest network. The character states of 238 characters could be explained by single nucleotide substitutions, while 18 characters were identified as being nonhomologous fragments expressing homoplastic similarities. Two examples of homoplastic similarity from Table 2 are the 2800 bp fragment seen in *Ava*II digestions of *O. tshawytscha* and *S. (Salmo) trutta* mtDNA and the 1210 bp fragment found in *Fnu*DII digestions of *S. (Parasalmo) gairdneri* and *S. fontinalis*. Since accurate genetic interpretation of these fragments is not possible with the available data, they were deleted from the network. The final resolved Wagner network is given in Fig. 4; numbers along branch lengths indicate the number of character state changes (roughly indicative of evolutionary distance). The position of the common ancestor to all four species is indicated by the arrowhead and allows the network to be interpreted as a cladogram.

A phenogram based on the sequence divergence estimates between the species is given in Fig. 5. The mtDNA of *S. (Parasalmo) gairdneri* is estimated to have diverged from the mtDNA of *O. tshawytscha* by 3.4%, *S. (Salmo) trutta* differ from the Pacific salmonids by 7.2%, and the mtDNA of *S. fontinalis* is estimated to have diverged by approximately 10%.

Discussion and Conclusions

Our cladistic interpretation (Fig. 4) indicates phylogenetic relationships in full accord with those suggested by comparative morphology, embryology, karyology, biogeography, and protein analyses (Rousenfell 1962; Behnke 1968, 1979; Utter et al. 1973; Okazaki 1974; Pavlov 1980; Gorshkov and Gorshkova 1981; F. W. Allendorf, Dep. Zool., Univ. Montana, Missoula, MT 59812, pers. comm.). Analysis of mtDNA supports the close relationship of *Oncorhynchus* and *Salmo (Parasalmo)* and their status as the sister group to *Salmo (Salmo)*. Thus, if nomenclature is intended to reflect phylogenetic relationship, it is arguable that *Parasalmo* should be accorded coordinate taxonomic rank as *Oncorhynchus* (see Valdykov 1963; Behnke 1979).

Our findings that 31% (18 of 58) of the phylogenetically informative characters may be the result of multiple nucleotide

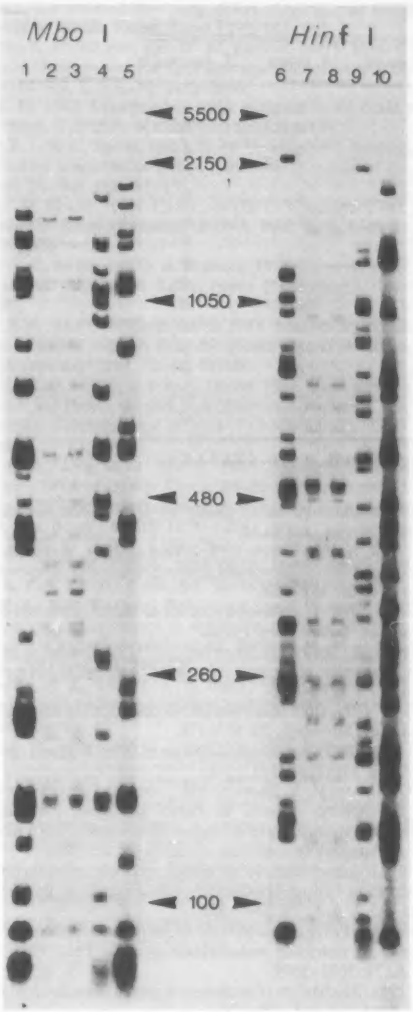

FIG. 3. Mitochondrial DNA fragment patterns produced by digestion with either *Mbo*I or *Hin*fI, restriction endonucleases that recognize four base sequences. Patterns 1 and 6: rainbow trout; 2, 3, 7, and 8: chinook salmon; 4 and 9: brown trout; 5 and 10: brook trout. The numbers in the middle are the sizes (bp) of PM2 phage DNA digested with *Hin*dIII.

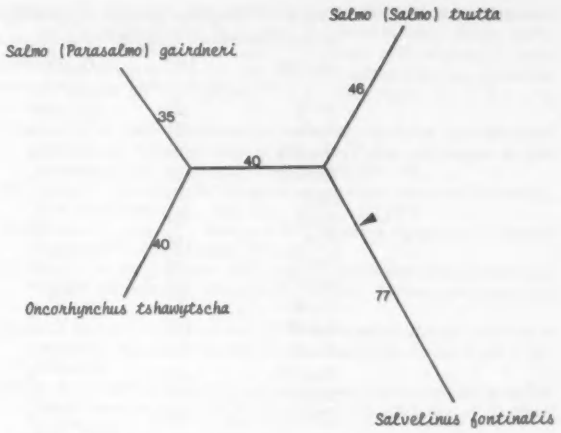

FIG. 4. Wagner network representing the most parsimonious arrangement of the four salmonid species' mtDNAs. Patristic distance between species represents the number of fragment differences between their mtDNAs. The arrowhead indicates the most likely root for the network (as described in the text).

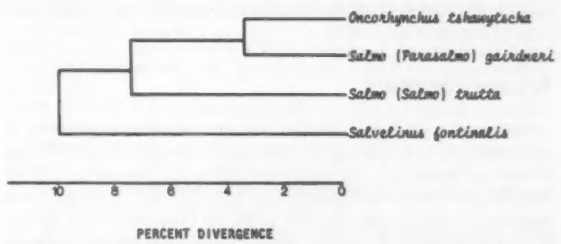

FIG. 5. Phenogram showing estimated percent sequence divergence among the mtDNAs of four salmonid species. Clustering was done using the UPGMA method (Sneath and Sokal 1973).

substitutions within the same restriction site is not surprising in light of the sequencing work of Brown and Simpson (1982) and Brown et al. (1982). These authors have found that not only does mtDNA evolve more rapidly than nuclear DNA but that there is an evolutionary bias in nucleotide substitutions in mtDNA strongly favoring transitions over transversions. One of the implications is that character state reversals, parallelism, and convergent events are increasingly likely to be found as one examines the mtDNA of more divergent taxa.

Another potential effect of this bias towards transitions is on estimates of sequence divergence. Although the comparative fragment method may give an underestimate when actual sequence divergence exceeds 5% (George 1982), the correspondence between estimated and actual values is generally good (Brown et al. 1982). We may therefore be relatively confident in the sequence divergence estimates presented here.

Most vertebrate species studied for mtDNA variation to date show that individuals within a species differ by 0–3% in mtDNA sequence (Avise et al. 1979a, 1979b; Brown and Simpson 1981; Ferris et al. 1981a, 1982; Yonekawa et al. 1981). Although our study has concentrated on between-species comparisons, the magnitude of the variation found must be put into perspective by consideration of within-species variation. The two chinook salmon had the same mtDNA pattern and in a recent survey of 14 skipjack tuna (*Katsuwonus pelamis*), the sequence variation was estimated to be less than 1% (Graves et al. 1984). A detailed examination of mtDNA variation within populations of salmonids must await the future. Nonetheless, it is interesting to note that our smallest sequence divergence value between mtDNAs, 3.4% between rainbow trout and chinook salmon, is within the range of values between mammalian conspecifics and congenerics (Brown and Simpson 1981; Ferris et al. 1981a, 1983; Lansman et al. 1981).

Since mitochondria are autonomous organelles that, at least in higher animals, do not undergo recombination, are maternally inherited, and experience a high mutation rate, they represent an independent record of evolution. Use of restriction endonucleases to partially sequence mtDNA yields large data sets that contain many diagnostic characters, thus giving the

TABLE 3. Fragment patterns of mitochondrial DNA found in four salmonid species.

Enzyme	(*n*)[a]	*O. tshawytscha*	*S. gairdneri*	*S. trutta*	*S. fontinalis*
*Sac*II	(6)	a	a	a	a
*Sac*I	(6)	a	a	a	b
*Hin*dIII	(6)	a	a	b	c
*Bam*HI	(6)	a	a	b	c
*Bgl*I	(6)	a	b	c	d
*Bgl*II	(6)	a	b	c	d
*Pst*I	(6)	a	b	c	d
*Pvu*II	(6)	a	b	c	d
*Xba*I	(6)	a	b	c	d
*Eco*RI	(6)	a	b	c	d
*Hinc*II	(6)	a	b	c	d
*Ava*II	(5)	a	b	c	d
*Fnu*DII	(4)	a	b	c	d
*Mbo*I	(4)	a	b	c	d
*Hinf*I	(4)	a	b	c	d

[a]*n* = number of nucleotides within recognition site (e.g. *Sac*II, *n* = 6, CCGCGG).

geneticist/population biologist a powerful new tool to examine evolution and population structure. Not only may this method help resolve many systematic problems, but it may aid the fishery scientist in identifying stocks of commercially important fishes (Ihssen et al. 1981).

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Spatial Distribution of Plankton in Enclosures of Three Sizes

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Stephenson, G. L., P. Hamilton, N. K. Kaushik, J. B. Robinson, and K. R. Solomon. 1984. Spatial distribution of plankton in enclosures of three sizes. Can. J. Fish. Aquat. Sci. 41: 1048–1054.

The horizontal distribution of plankton was studied in large (1000 m^3, 16 m diameter, 5 m deep), medium (5 × 5 × 5 m deep) and small (2 × 2 × 5 m deep) enclosures in a 10.3-ha mesotrophic lake in southern Ontario. Zooplankton population estimates from samples collected along distance gradients in the small and medium enclosures varied slightly but no consistent patterned distribution was present. However, the large enclosures possessed a distinctive edge zone that extended about 1.0 m from the walls. On two of the three sampling times there were significantly more macrozooplankton and/or fewer microzooplankton in the edge zone. However, on no occasion were there fewer macroplankton or more microzooplankton in the edge zone. Although macrozooplankton, as a group, may be significantly more numerous in the edge zone ($P \leq 0.05$), individual species within this group exhibited both positive and negative responses. There was no defined edge zone in any enclosure with respect to phytoplankton density or biomass. Definition of the spatial distribution of organisms is essential to maximize precision of population estimates when using enclosure systems for ecotoxicological studies.

Les auteurs ont étudié la répartition horizontale du plancton dans des enclos de volume grand (1000 m^3, 16 m de diamètre, 5 m de profondeur), intermédiaire (5 × 5 × 5 m de profondeur) et petit (2 × 2 × 5 m de profondeur), situés dans un lac mésotrophe de 10,3 ha, dans le sud de l'Ontario. Les estimations de la population de zooplancton effectuées à partir d'échantillons recueillis le long de grandients de distance dans les enclos de taille petite et intermédiaire variaient légèrement mais aucune répartition ordonnée n'était présente. Toutefois, une zone distinctive qui s'étendait jusqu'à environ 1,0 m des parois s'est manifestée dans les grands enclos. Au cours de deux des trois levés, il y avait nettement plus de zooplancton et moins de microzooplancton dans cette zone, mais jamais le contraire. Même si le macrozooplancton, comme groupe, pouvait être significativement plus abondant sur les bords ($P \leq 0{,}05$), les espèces individuelles au sein d'un groupe démontraient des réactions positives et négatives. Aucune zone en bordure définie, quant à la densité ou à la biomasse du phytoplancton, n'était présente dans les enclos. La définition de la répartition spatiale des organismes est essentielle à la précision des estimations de la population quand on utilise des systèmes d'enclos pour les études écotoxicologiques.

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Laboratory toxicity tests are not suitable for predicting the impact of a toxicant on an entire community (report by the Committee to Review Methods for Ecotoxicology 1981). Such studies usually involve assessing the impact of a toxicant on a single species or at best a few species at one time and do not provide adequate assessment of an impact on the interactions that may occur among different species.

To overcome such shortcomings, considerable effort has been directed in recent years toward the use of in situ enclosures, or limnocorrals, for assessing the effects of pollutants at the community level (Kuiper 1977; Grice and Menzel 1978; Davies and Gamble 1979; Steele 1979; Smith et al. 1980; Grice and Reeve 1982). However, before these test systems become acceptable procedure and are standardized, it is necessary to understand the distribution of naturally occurring plankton populations within the enclosed water column. In this paper we present information on the spatial distribution of the pelagic plankton in enclosures of three sizes.

Materials and Methods

Three large (1000 m^3, 16 m diameter, 5 m deep) and three medium (125 m^3, 5 × 5 × 5 m deep) enclosures made of yellow nylon-reinforced PVC and three small (20 m^3, 2 × 2 × 5 m deep) polyethylene enclosures located in a 10.3-ha mesotrophic lake (Lake St. George, Ontario, 41°51′25″N, 77°25′30″W) were used for this study. Construction and installation of these limnocorrals was similar to that described by Solomon et al. (1980). Walls of the limnocorrals were dropped within a 5-d period and anchored firmly into the sediment, effectively isolating comparable sections of the lake ecosystem from the water surface to the lake sediment.

Zooplankton were collected with a vertically integrating 150-mm (O.D.) tube sampler (Solomon et al. 1982), which isolated a column of water from the surface to approximately 15 cm above the sediment, and concentrated by filtering the water through 30-μm Nytex mesh. The sample was immediately preserved in a sucrose-buffered 4% formalin solution (Haney and Hall 1973). In the laboratory the sample was placed in an octagonal Plexiglas graduated container and evenly mixed with a magnetic stirring bar. A 5-mL subsample was pipetted into a graduated circular Plexiglas counting wheel (modified from Ward 1955) mounted on a stereomicroscope, and the macrozooplankton (Cladocera and Copepoda) were identified and enumerated. The microzooplankton (Rotatoria) were identified and enumerated from a 2-mL subsample in a Sedgewick Rafter

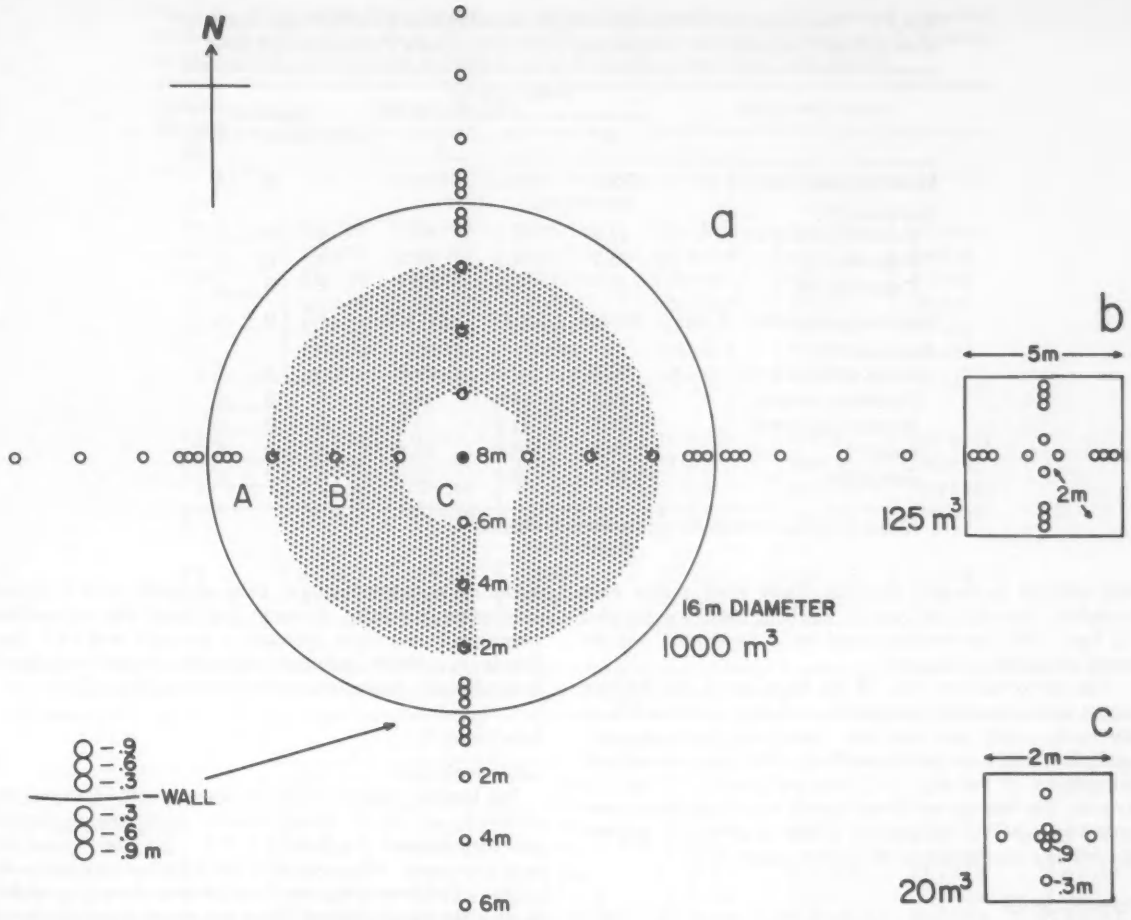

FIG. 1. Sampling sites in the enclosures. (a) large corral (A, edge zone; B, middle zone; C, central zone); (b) medium corral; (c) small corral.

Cell using phase contrast microscopy. A minimum of 300 organisms were counted (usually one or two subsamples). The zooplankton were identified according to Brooks (1957), Edmondson (1959), Chengalath et al. (1971), Grothe and Grothe (1977), Pennak (1978), and Smith and Fernando (1978).

For phytoplankton, integrated samples (3.5 m deep) were collected using a 25 mm (O.D.) plankton sampler (Solomon et al. 1982). A 115-mL sample from each column of water was preserved with Lugol's solution. In the laboratory a minimum of 350 individuals were counted using Utermohl's technique (Lund et al. 1958). Samples of 1–7 mL were counted depending on the density of the plankton. All enumerations were converted to number of organisms per litre, and phytoplankton biomass values (milligrams per cubic metre) were calculated using the method described by Janus and Duthie (1979).

All plankton samples were collected between 11:00 and 16:00. The weather conditions on the three zooplankton sampling days were very similar: generally overcast with little or no wind and a light drizzle on 10 June and 17 September.

Experiment I

Because planktonic organisms were observed in what was suspected to be abnormally large numbers along the walls of the 1000-m^3 circular enclosures, we decided to investigate the horizontal distribution in these limnocorrals. One large corral was arbitrarily divided into three concentric zones, A, B, and C (Fig. 1a), and 10 zooplankton samples were collected randomly from each zone on 10 June 1982 and processed in the laboratory. The data were analyzed using a nonparametric Kruskal–Wallis one-way analysis of variance by ranks (Elliot 1971).

Experiment II

Triplicate corrals of each size were sampled on 27 July 1982. One zooplankton sample was collected at each distance from the corral wall as shown in Fig. 1a, 1b, and 1c along north–south and east–west transects. The preserved samples were processed in the laboratory as mentioned earlier. A parametric two-way analysis of variance was performed on the transformed ($\log_{10}$) data from each large and medium corral. Duncan's multiple range method was used to delineate differences. An edge versus centre comparison of the data from the small enclosures was made with a nonparametric Mann–Whitney *U*-test (Elliot 1971).

One phytoplankton sample was also collected from one large and one medium corral at distances shown in Fig. 1a and 1b along north–south (large enclosure) and east–west (both large

TABLE 1. Mean density of microzooplankton and macrozooplankton and percentage abundance of the dominant zooplankton in each sampling zone for Experiment I (10 samples per zone).

	Sampling zone			Significant differences ($P < 0.05$)[a]		
	A	B	C			
Microzooplankton (no/L ± SE)	376±13	506±21	626±41	A	B	C
Abundance (%)						
Keratella cochlearis	23.8	35.1	41.1	A	B	C
Polyarthra spp.	25.7	32.5	42.5	A	B	C
Conochilus spp.	20.7	36.8	42.5	A	B	C
Macrozooplankton (no/L ± SE)	465±24	356±7	401±32	A	B	C
Abundance (%)						
Ceriodaphnia lacustris	32.7	37.5	29.9	A	B	C
Eubosmina coregoni	19.4	41.4	39.2	A	B	C
Bosmina longirostris	75.3	11.1	13.7	A	B	C
nauplii	20.3	35.9	43.8	A	B	C
copepodites	38.1	29.5	32.4	A	B	C

[a]Zones connected by lines are not significantly different.

and medium enclosures) transects. Three small corrals were sampled at the edges and centres. The large corral was sampled 22 June 1982, the medium corral on 17 September, and the small corrals on 24 August.

The phytoplankton data for the large corral and medium corral were statistically analyzed in two steps: a comparison of the north, south, east, and west transects using a parametric analysis of variance (Steele and Torrie 1960) and a subsequent comparison of the edge (<1.0 m) and centre (1–7 m) data (t-test). The data for the 20-m^3 corrals were examined in two steps: an ANOVA comparison of the three corrals and the pooled data comparison of the edge to centre data.

Experiment III

To determine whether the distribution of zooplankton observed within the large enclosures in the above experiment persisted at turnover, the zooplankton sampling regime detailed in experiment II was repeated on 17 September 1982 in one large enclosure. The data were analyzed with a Mann–Whitney U-test comparison of edge versus centre sample estimates. A comparison was made with the open lake zooplankton community by sampling along four transects radiating outward from the limnocorral wall into the lake (Fig. 1a).

Results

Experiment I

There were significantly more macrozooplankton and significantly fewer microzooplankton ($P \leq 0.05$) in zone A, the area nearest the wall of the enclosure (Table 1). Zooplankton numbers did not differ significantly between the middle (B) and centre (C) zones. The species of rotifers comprising >80% of the total rotifer populations are listed in Table 1. These rotifers were consistently dominant in the same relative proportions in all zones. Ninety percent of the macrozooplankton consisted of adult *Ceriodaphnia lacustris* Birge, *Eubosmina coregoni* (Baird), *Bosmina longirostris* (O. F. Muller), and the immature stages nauplii and copepodites (Table 1). However, unlike the microzooplankton, there appeared to be a species distribution specificity. *Bosmina longirostris* and copepodites were significantly more abundant in the edge zone (A). Conversely, *E. coregoni* and macrozooplankton nauplii were found in significantly greater proportions in zones B and C.

Experiment II

Large enclosures

The transect counts (Table 2) show clearly a significant (ANOVA, $n = 28$, $P \leq 0.05$) decline in the total number of macrozooplankton at a distance > 0.9 m from the walls of the large enclosures. Although there was a gradual increase in the number of microzooplankton along the same distance gradient, no edge was clearly defined. There was no consistent significant variation among the four radii within each corral. It is clear that the "edge effect" is due to the large numbers of *B. longirostris* and *C. lacustris* within 1.0 m of the enclosure wall (Table 3). *Daphnia galeata mendotae*, which increases numerically towards the end of the summer, was significantly more abundant in the centre zone.

There was no significant difference in total numbers per litre of phytoplankton among the transects (ANOVA, $n = 28$, $P = 0.35$). The edge to centre comparison of the major phytoplankton groups (Chlorophyta, Chrysophyta, and Cryptophyta) showed no difference in total numbers (t-test, $n = 28$, $P > 0.50$) (Table 4).

The total phytoplankton community biomass was significantly higher in the south as compared with the east and west transects (t-test, $P < 0.04$). Three species, *Chlorococcale* sp. a, *Chlorella vulgaris*, and *Gleocystis planktonica*, of the group Chlorophyta, which comprised from 77 to 96% of the standing crop, were responsible for the elevated biomass in the south transect. The Cryptophyta had a community standing crop greater in the west than east. This difference was caused by the higher biomass of *Cryptomonas erosa* in the west transect. The Chrysophyta were distributed evenly among the four transects; however, their contribution to the community standing crop was less than 0.2% (Table 5).

The edge to centre comparison showed no significant difference in total biomass (t-test, $P > 0.10$). This uniformity

TABLE 2. Mean number (four samples ± SE) of zooplankton per litre in samples collected at specified distances from the wall of each of the large, medium, and small enclosures for experiment II. Sampling distances joined by vertical lines or followed by the same letter are not significantly different.

Distance from wall (m)	Macrozooplankton			Microzooplankton		
	1	2	3	1	2	3
			Large corrals			
0.3	343±58	369±67	306±21 a	594±50 ef	507±67	482±49
0.6	344±32	416±81	231±21 b	611±83 ef	476±19	504±35
0.9	438±88	314±77	199±25 bc	537±153 e	500±25	447±40
2.0	141±14	141±17	145±9 d	695±42 ef	539±34	552±47
4.0	155±13	144±16	148±11 dc	754±67 f	589±34	557±31
6.0	157±7	150±3	156±9 dc	730±41 f	567±17	624±32
8.0	151±14	168±10	162±15 dc	695±65 f	607±20	540±17
			Medium corrals			
0.3	279±55	109±5	101±15	177±51	462±82	1091±133
0.6	246±41	117±3	114±10	123±5	690±153	1062±93
0.9	232±53	126±11	137±13	213±61	638±139	1149±181
2.0	261±33	171±16	142±21	152±18	824±42	1113±50
			Small corrals			
0.3	42±3	273±29	78±11	383±16	164±29	286±48
0.9	47±4	370±42	113±29	439±37	128±19	298±23

TABLE 3. Mean numbers per litre ($n = 4$) of within-corral replicates (±SD) of the most abundant macrozooplankton at various distances from the wall of the large enclosure. Values followed by the same letter are not significantly different at $P \leq 0.05$.

Species	Distance from wall (m)						
	0.3	0.6	0.9	2.0	4.0	6.0	8.0
Bosmina longirostris	174.4±51 a	126.9±54 a	78.1±34 a	15.8±1 b	11.4±9 b	5.1±2 b	5.2±1 b
Ceriodaphnia lacustris	25.3±8 a	17.3±8 a	14.7±4 a	5.9±2 b	5.4±3 b	4.4±1 b	4.8±2 b
Daphnia galeata mendotae	3.8±3 a	1.8±1 a	7.1±4 a	27.1±8 b	25.6±6 b	35.1±8 b	32.4±15 b
Diaptomus oregonensis	3.6±2 a	3.0±2 a	6.0±3 a	4.1±2 a	4.2±1 a	5.2±3 a	6.3±3 a
Immatures	36.6±10 a	33.8±13 a	36.0±12 a	39.2±6 a	43.3±6 a	45.7±7 a	49.1±13 a

between edge and centre was expressed by all the major groups (Table 4).

Medium enclosures

There were no significant differences in total and group numbers of both phytoplankton (Table 5) and zooplankton (ANOVA, $n = 16$, $P > 0.05$) between transects within enclosures, and no difference between edge and centre samples for total and group numbers of phytoplankton (Table 4). There was a significant increase in density of macrozooplankton at 2.0 m in one corral, and in two of the three corrals there was a gradual increase in density of macrozooplankton along the distance gradient, perhaps indicating a trend toward two zones (Table 2). There was no significant variation in estimates of microzooplankton along the distance gradient.

Small enclosures

Estimates of zooplankton density showed no significant differences in terms of edge versus centre for both zooplankton groups (Mann–Whitney U-test, $P > 0.05$) (Table 2), and no significant variation was observed among the three corrals with respect to total numbers of phytoplankton and biomass (ANOVA, $P = 0.40$, $P = 0.58$). The pooled corral data showed no difference (with respect to total and group numbers of phytoplankton, and biomass) between edge and centre (t-test, $P > 0.50$) (Table 4).

Experiment III

There was no significant difference between edge and centre estimates of macrozooplankton; however, the density of microzooplankton was significantly larger in the centre zone (Mann–Whitney U-test, $P \leq 0.05$). Analysis of the four 6-m transects radiating from the enclosure wall into the lake showed no significant variation between transects or along the transects.

Discussion

Intuitively it appears that larger enclosures better approximate the natural ecosystem because they can successfully support more trophic levels (Parsons 1978) and greater population densities (Case 1978) over a longer period of time. Steele (1979) correlated increased spatial heterogeneity with increasing enclosure size. Similarly, Takahashi et al. (1975) and Grice et al. (1977) observed increased patchiness in the plankton distribution of large enclosures. Comparable results were obtained in the present study. The phytoplankton density was

TABLE 4. Summarized comparisons between the edge and centre of the

	1000 m³				
	Edge		Centre		
	n	$x \pm$ SE	*n*	$x \pm$ SE	*n*
Nos. ($\times 10^6$)/L					
Total	12	201.3±14.6	16	196.1±13.6	2
Chlorophyta	12	193.3±14.7	16	187.4±13.1	2
Chrysophyta	12	4.4±8.7	16	5.8±0.9	2
Chryptophyta	11	2.2±0.4	16	2.5±0.3	2
Biomass (mg/m³)					
Total	12[a]	918.9±132.0	16	706.6±41.7	2
Chlorophyta	12[a]	817.5±133.3	16	596.4±39.9	2
Chrysophyta	12	7.2±1.4	16	7.4±1.3	2
Chryptophyta	11	80.7±12.9	16	80.2±8.8	2

[a]Analysis was done on ln transformed data to meet the ANOVA requi

TABLE 5. Summarized comparisons for the different transects in the medium and

	Large enclosure			
	North	South	East	Wes
Nos. ($\times 10^6$)/L				
Total	197.7±23.0	228.4±25.8	182.1±9.2	185.0±
Chlorophyta	187.6±23.0	219.6±25.3	176.5±8.7	176.1±
Chrysophyta	5.7±0.9	6.0±2.0	3.8±0.6	5.4±
Cryptophyta	2.8±0.2	2.3±0.5	1.6±0.3	2.4±
Biomass (mg/L³)				
Total	873.7±99.2	1022.9±204.6	608.1±32.1	673.1±
Chlorophyta	766.8±98.6	919.2±207.5	551.3±32.6	527.4±
Chrysophyta	7.7±2.0	8.2±2.8	6.1±1.0	7.3±
Cryptophyta	88.0±11.1	75.0±16.4	48.7±6.8	105.3±

re of the three different corral sizes, using phytoplankton numbers and biomass.

	125 m³				20 m³			
	Edge		Centre		Edge		Centre	
	n	$x \pm$ SE	*n*	$x \pm$ SE	*n*	$x \pm$ SE	*n*	$x \pm$ SE
6	2	17.8±0.8	3	17.0±1.0	3	31.9±7.1	3	31.2±1.1
1	2	15.5±0.8	3	14.9±1.0	3	8.6±3.2	3	7.8±3.0
	2	—	3	—	3	3.9±1.6	3	2.9±1.4
	2	1.9±0.2	3	1.8±0.1	3	17.6±9.9	3	20.0±8.3
7	2	219.0±1.0	3	193.7±40.1	3	1248.3±108.9	3	1582.2±363.7
9	2	64.9±10.9	3	67.7±10.8	3	98.9±19.3	3	157.9±111.1
	2	—	3	—	3	14.2±7.4	3	28.7±18.7
	2	123.8±3.0	3	114.6±19.1	3	787.1±313.1	3	887.5±338.7

/A requirements.

ium and large enclosures, using phytoplankton numbers and biomass.

ıre			Medium enclosure		
West	ANOVA or Duncan's range test		North	South	*t*-test
185.0±15.1	No difference, $P = 0.35$		17.0±1.6	17.9±0.9	No difference, $P > 0.50$
176.1±14.7	No difference, $P = 0.37$		14.8±1.5	15.7±1.0	No difference, $P > 0.50$
5.4±1.1	No difference, $P = 0.68$		—	—	
2.4±0.6	No difference, $P = 0.11$		1.9±0.2	1.8±0.8	No difference, $P > 0.50$
673.1±50.8	E W N S		207.5±10.5	186.0±34.0	No difference, $P > 0.50$
527.4±41.2	W E N S		62.2±13.6	61.2±7.2	No difference, $P > 0.50$
7.3±1.2	No difference, $P = 0.91$		—	—	
105.3±13.5	E S N W		130.4±9.5	102.0±24.8	No difference, $P > 0.50$

TABLE 6. Densities of macrozooplankton in the large enclosures corrected for volume.

Corral	Mean no. of organisms/L: Based on samples: from edge[a]	Mean no. of organisms/L: Based on samples: from centre[b]	Mean no. of organisms/L: Corrected by volume	% error if estimates based on: edge samples only	% error if estimates based on: centre samples only
1	375	151	203	85	26
2	366	150	201	82	25
3	245	153	174	41	12
Mean				69	21

[a]Correction factor 0.23.
[b]Correction factor 0.77.

random within an enclosure with no edge to centre difference; however, biomass differences in the large corral were indicative of increased patchiness.

In the large enclosures an edge zone existed with respect to distribution of zooplankton, and depending on the sampling date, it was characterized by having significantly more macrozooplankton and/or significantly fewer microzooplankton. Within the macrozooplankton group, the defined distribution was species specific and temporally variable (Tables 2 and 3) whereas species of microzooplankton were present in each zone in the same relative proportions. We do not know why this "zonation" occurred; however, there may be a number of possible contributing factors. The macrozooplankton aggregating in the edge zone may be grazing on food particles (detritus, bacteria, and algae) associated with periphytic growth on walls of the enclosure. Although these organisms are thought to be limnetic, they may be found in both littoral and benthic zones (McNaught et al. 1980). It has been suggested by Kwick and Carter (1975) that during periods of food shortage, *Bosmina* sp. retreat to benthic areas to exploit sedimented detrital particles. This pattern was not observed in the medium and small limnocorrals, which may not be surprising given the relative volumes of the edge zone within each size of enclosure. The edge zone constitutes 23% of the total volume of a large corral, 64% of a medium corral, and 100% of a small corral. No edge zone was present on the "outside" or open lake side of the large corral, indicating perhaps that it is not merely the presence of a wall that is important but the enclosure shape and/or size. Circular currents could be easily induced by winds blowing from any direction, thus affecting the distribution of zooplankton within the large enclosures. The square small and medium enclosures may not have the same kind of currents; however, this was not investigated in the present study.

It is also possible that the distribution of zooplankton within the large corrals could be attributed to the differential behavioral response of zooplankton to enclosure. The causal factors for a behavioral response could possibly include a combination of temperature, turbidity, and light intensity and wavelength. The latter has been shown (Wilson 1982) to be a significant factor for phototaxis of zooplankton. The solid yellow colour of the walls of limnocorrals may affect the incident solar radiation below the water surface due to differential absorption of wavelengths and perhaps result in entrainment of photosensitive zooplankton species within the narrow edge zone. Hailman and Jaeger (1976) suggested that animals tend to maintain themselves close to species-specific optimum intensities to maximize input of visual information. Perhaps this is true for those organisms aggregating in the edge zone. Future research is necessary to elucidate causal factors of this distribution.

The proportional contributions of the edge and centre zones to the total volume of the large enclosure are 23 and 77%, respectively. Therefore, to improve the precision of estimates of zooplankton density, these zones should either be sampled separately or a number of samples pooled to represent the relative contribution of each zone. If a population density is based on a sample taken from the edge zone then the estimate of the aggregating organisms will be inflated, and the organisms that are significantly less abundant in the zone will be underestimated. Similarly, sampling from the centre zone alone would result in erroneous estimates of zooplankton density. For example, when the macrozooplankton densities in Table 2 are adjusted to correct for relative volume, more precise population estimates are attained (Table 6). Without this correction the population estimates are either overestimated by an average of 69% or underestimated by an average of 21%.

Beers et al. (1977) used similarly sized enclosures (1300 m^3) in a marine ecosystem and examined horizontal distribution at distances of 1.0, 3.0, and 5.0 m from the enclosure wall. No sustained pattern of abundance was detected using rank order analysis (Williams et al. 1977). The diameter of their 1300-m^3 enclosures was 10 m whereas our 1000-m^3 limnocorrals were 16 m in diameter. It may be that this defined zooplankton distribution is a temporal phenomenon unique to freshwater enclosures of this magnitude; on the other hand, the "edge zone" may have existed in Beers' enclosures but was not detected by the sampling regime employed.

Our studies have identified a potential problem that all investigators using enclosures for toxicology studies must resolve. The distribution of organisms within enclosed water columns should be investigated prior to experimentation, and sampling strategies adapted to reduce error in estimates of population density.

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Timing of Spawning of Atlantic Herring (*Clupea harengus harengus*) Populations and the Match–Mismatch Theory

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Sinclair, M. and M. J. Tremblay. 1984. Timing of spawning of Atlantic herring (*Clupea harengus harengus*) populations and the match–mismatch theory. Can. J. Fish. Aquat. Sci. 41: 1055–1065.

Each population of Atlantic herring (*Clupea harengus harengus*) has its own seasonally fixed spawning period of a few weeks duration, but the mean spawning times of different populations differ substantially. The extant theory explains the population-specific timing of spawning relative to the plankton production blooms in the inferred larval distributional area. Support of this theory is evaluated, and found lacking, in the light of a recent "stock" hypothesis involving larval retention. The new hypothesis involves two constraints. First, the larvae of a discrete herring population develop within, and are thus adapted to, the specific oceanographic conditions of their larval retention area. Second, metamorphosis from the larval to juvenile form occurs primarily within a restricted period of the year (April to October). Given these two constraints, it is hypothesized that the timing of spawning of a herring population is a function of the time necessary to complete the larval phase and yet metamorphose within the acceptable seasonal envelope. Populations that have "good" larval retention areas can spawn in the spring and still metamorphose within the seasonal envelope. Populations with larval retention areas that are less "good" for larval growth have to spawn earlier to satisfy the two constraints. The implications of the hypothesis on the "match–mismatch" theory are briefly discussed.

Chaque population de hareng de l'Atlantique (*Clupea harengus harengus*) possède sa propre période fixe de fraie saisonnière, qui dure quelques semaines, mais le temps moyen de fraie de différentes populations varie considérablement. La théorie existante explique le temps de fraie spécifique à chaque population en termes de proliférations de plancton dans la zone de répartition des larves. Les auteurs évaluent les preuves de cette théorie de les trouvent faibles quand on tient compte de la nouvelle hypothèse du « stock », laquelle porte la rétention des larves et comprend deux contraintes. Premièrement, les larves d'une population isolée de hareng se développent dans les conditions océaniques déterminées de la zone de rétention des larves et y sont donc adaptées. Deuxièment, la métamorphose des larves en juvéniles a lieu surtout pendant une courte période de l'année (d'avril à octobre). Á partir de ces deux faits, les auteurs formulent l'hypothèse que la période de fraie chez une population de hareng est fonction du temps nécessaire à la réalisation de la phase larvaire et à la métamorphose, pendant la période saisonnière acceptable. Les populations qui possèdent de « bonnes » zones de rétention des larves peuvent frayer au printemps et les larves peuvent de métamorphoser en deçà de la période acceptable. Les populations dont les zones de rétention sont moins bonnes doivent frayer plus tôt afin de se soumettre aux deux contraintes. Les auteurs discutent des répercussions de l'hypothèse sur la théorie du « bien assorti » et du « mal assorti ».

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In the recent fisheries biology literature, the timing of spawning of marine fish species in north temperate waters is coupled with the generation of between-year variability in recruitment. Fish populations in such waters are hypothesized to spawn in relation to seasonal plankton blooms. The between-year differences in the timing of phytoplankton blooms in relation to the relatively fixed period of spawning have been proposed to explain the variance in year-class strengths. This concept has been given the status of a theory: the "match–mismatch" theory (Cushing 1975). Cushing stated, for example, that "the importance of the match/mismatch theory lies in showing how a fish stock can respond to climatic variation and climatic trends" (p. 232). Much of the recent research on larval fish ecology has been conducted in relation to this theory.

Research on Atlantic herring (*Clupea harengus harengus*) has played a key role in the development of these concepts. Here, the relevant observations on the timing of spawning of herring are reevaluated relative to recent developments in fisheries biology and oceanography. The hypothesis on the timing of spawning of herring is argued to be inconsistent with the accumulated observations, and a new hypothesis is presented. Finally, the implications on the match–mismatch theory are briefly discussed. To put the subject matter in context, it is fruitful first to review the developments that have led to the formulation of the match–mismatch theory and the importance of herring therein.

Development of the Match–Mismatch Theory

Five key steps can be identified in the development of the theory. By the 1950's it was well recognized by fisheries biologists in Europe that each of the many populations of

TABLE 1. Peak spawning periods for herring populations. The estimates are obtained from peak hatching data (estimated incubation time subtracted) or from peak catches from a spawning fishery. Time periods with an asterisk are derived by taking the midpoint of the spawning period. If more than 1 yr of observations are considered, this is indicated in parentheses.

Stock(s)	Peak period	Reference(s)
1. Clyde Sea	February 20–28	Parrish et al. 1959
2. Norwegian	February 18–March 18 (27 yr)	Dragesund et al. 1980
3. Minch	March*	Baxter 1958; Saville and Morrison 1973
4. Blackwater Estuary	April*	Wood 1981
5. Schlei Fjord–Kiel Bay	April*	Schnack 1972
6. Magdalen Island	May 9 (27 yr)	Ware and Henriksen 1978
7. Southwest Gulf of St. Lawrence	May 14–18 (27 yr)	Ware and Henriksen 1978
8. Chedabucto Bay	May*	Sameoto 1971
9. Southeast Gulf of St. Lawrence	May 29–June 6 (27 yr)	Ware and Henriksen 1978
10. St. Lawrence Estuary	June 15–July 7 (5 yr)	Able 1978; Auger and Powles 1980
11. Scots Bay	July20–August 3 (6 yr)	M. Sinclair, unpubl. data
12. Northwestern North Sea	August	Iles 1964; Saville 1978
13. Southern Gulf of St. Lawrence	July31–September 13 (27 yr)	Ware and Henriksen 1978
14. Southwest Nova Scotia	August 25–September 10 (5 yr)	Das 1968; M. Sinclair and T. D. Iles, unpubl. data
15. Grand Manan (historical)	July–September	Iles and Hatt 1980
16. Minch	September 5–25	McKay 1978
17. Banks–Dogger	September*	Zijlstra 1973; Cushing 1967
18. Manx	September	Bowers 1952
19. Mourne	Late September–October	Bowers 1980
20. Coastal Gulf of Maine		
Eastern section	September 15–October 17	Graham et al. 1972
Western section	October 1–21 (5 yr)	
21. Jeffrey's ledge	September 29–October 25 (2 yr)	Boyar et al. 1973a; Pecci 1975
22. Donegal	October	McArdle 1982
23. Georges Bank	October 5–23 (8 yr)	Lough et al. 1980
24. Nantucket Shoals	October 12–November 2 (8 yr)	Lough et al. 1980
25. Dunmore	(September–October, December–January)	Molloy 1979
26. Downs	December	Iles 1964; Zijlstra 1973
27. Plymouth	January	Cushing 1967; Lebour 1921b

herring had its own seasonally fixed spawning period of several weeks but that the mean spawning time of different populations differed substantially (Table 1; Fig. 1). The explanation of this phenomenon was an important area of speculation. Iles (1964) attempted to explain the timing of spawning of the herring populations in the coastal waters of the United Kingdom in relation to the specific characteristics of plankton seasonal cycles in the adult feeding areas. He concluded, however, that observed differences in plankton seasonal cycles in the adult feeding areas could not account for the full range of the timing of spawning of the different herring populations. Following this lead, Cushing (1967) linked similar data on phytoplankton cycles in inferred larval drift areas (rather than the adult summer-feeding areas) to the respective periods of spawning. Since the larval drift areas for the different spawning populations were not well described, the author was not unduly constrained in matching phytoplankton seasonal cycles in diverse geographic areas in relation to spawning locations that were at some distance from the inferred larval drift environment. The hypothesis that he generated from the analysis, the link between the timing of spawning of herring populations and seasonal phytoplankton blooms, was sufficiently attractive to gain general acceptance even though empirical support was weak.

The third step was the generalization that several other north temperate fish species (*Gadus morhua*, *Pleuronectes platessa*, *Oncorhynchus nerka*) have a fixed period of spawning with limited between-year variability in the timing (Cushing 1969). Next came a general model linking a fixed period of spawning for marine fish to variable timing of phytoplankton blooms due to stabilization/destabilization of the water column (Cushing 1973). In the model, variability in blooms was generated by a modified "critical depth" formulation. Variable wind stress generated differences in the timing of the development of sufficient density stratification in the water column in the spring (or destratification in the autumn) to permit logarithmic growth of the mixed phytoplankton populations (a bloom). Such variability in primary production, and the subsequent generation of food for larval fish (copepod nauplii and copepodites), combined with the presumed relatively fixed period of first feeding for a given population, generated variable larval survival. The final step was the fuller elaboration of these concepts into the attractively phrased "match–mismatch theory" (Cushing 1975, p. 180–183). This theory links three important

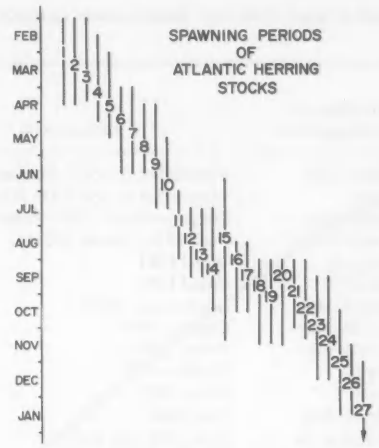

FIG. 1. Spawning periods of Atlantic herring populations. Populations corresponding to numbers given in Table 1.

characteristics of marine fish populations (timing of spawning, annual recruitment variability, and long-term changes in fish abundance) to a simple but successful model relating phytoplankton dynamics to physical properties of the water column: the critical depth model of Sverdrup (1953).

There are several important points to note in this theory. First, the match or mismatch is specifically associated with the results of the variable timing of phytoplankton blooms due to seasonal density stratification changes, and larval food availability is what ultimately causes the variable larval survival. Repeatedly in recent studies the link between spawning time and the primary production cycle has not always been explicitly made when using the phrase "match–mismatch." Instead, the emphasis has been on the co-occurrence of larvae with specific prey items or the influence of local environmental conditions thereon (e.g. Frank and Leggett 1982; Sherman et al. 1981). The theory, however, as developed, is not a match or mismatch between larval events and any number of different oceanographic, climatic, or biological phenomena. There are constraints on what constitutes a match or a mismatch, without which the theory would not have had either its elegance or appeal. Second, the theory assumes that larval drift is defined in relation to residual surface layer circulations. Third, the key empirical observations on which the ideas are developed are associated with herring populations, and the observations are evaluated based on the above-mentioned two inferences. In spite of the limited empirical support for the theory, but perhaps because fisheries biology was in need of integrating concepts to explain the remarkably diverse observations on herring and other fish species, the theory has gained wide acceptance.

Implications of Recent Observations

It has recently been hypothesized that the very existence of herring populations, as well as the specific location of spawning and the mean population size, is a function of the location and extent of geographically predictable larval retention areas (Iles and Sinclair 1982).

The term "larval retention" has proven to be provocative, but perhaps usefully so. Hypotheses involving larval drift generally include the concept that larvae are transported passively by the residual currents from the spawning location to the preferred nursery area where metamorphosis occurs. By larval retention it is inferred that the larvae actively maintain discrete distributions in relatively fixed geographical locations by, in the case of Atlantic herring, vertically migrating in relation to the depth differences in current speed and direction (Fortier and Leggett 1983; Graham 1972). It is not inferred that the larvae are retained passively by the circulation features of the larval distributional area, but rather by an active behavioural response to the physical regime. Clearly there is a question of scale to be considered. Individual larvae must indeed drift horizontally during their vertical migrations, but nevertheless the populations are observed to maintain a discrete distribution in a relatively fixed geographic location over several months. Many earlier studies of the larval phase of herring (e.g. Fulton 1906; Masterman 1895; Meyer 1878) implicitly assumed in the original treatment of the data that a discrete population was being sampled over time and thus that the larvae were retained through much of the larval phase rather than drifting with the residual current.

The Iles and Sinclair (1982) stock hypothesis was based, to a large degree, on the repeated observations in the western Atlantic that the larvae of herring populations do not drift passively in response to the residual surface layer circulation, but rather retain themselves for at least several months in relatively limited geographical areas having specific oceanographic characteristics. In tidally energetic seas the larval distributional areas subsequent to spawning are coincident with areas characterized by vertically well-mixed water columns. Importantly, almost all herring populations that spawn in tidally energetic seas are late summer–autumn spawners. There are also many herring populations in seas that are not tidally energetic, and it was argued that their larvae are also retained but in relation to different physical factors. Many of these populations are spring spawners. Both from field studies (Fournier et al. 1984; Pingree et al. 1975, 1976) and theoretical work (Pingree 1978), generalizations can be drawn concerning the seasonal cycles in phytoplankton production that are associated with tidally well-mixed areas, well-stratified areas, and transition zones between these two types (Fig. 2). Tidally well-mixed areas are shown to be characterized by relatively low phytoplankton production at all seasons, whereas stratified areas are characterized by spring and autumn blooms with low production through the summer. The transition areas are highly productive throughout the season and thus do not have autumn

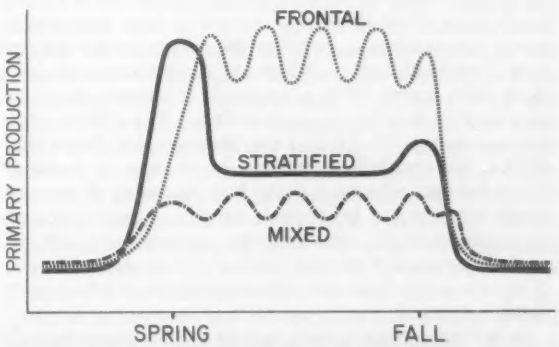

FIG. 2. Schematic representation of seasonal cycles of primary production in frontal, mixed, and stratified areas.

TABLE 2. Herring larval phase duration and seasonability of metamorphosis (assumed to begin at 40 mm). Retention area characteristics from Garrett et al. (1978), Pingree and Griffiths (1978, 1980), or references cited.

Stock(s)	Larval retention area characteristic	Duration of larval phase (mo)	Month(s) of metamorphosis	Reference(s)
5. Schlei Fjord–Kiel Bay	Stratified	2.5–3	June–July	Marshall et al. 1937; Schnack 1972
1. Clyde Sea	Stratified	3	June	Marshall et al. 1937; De Silva 1973
2. Norwegian	Stratified	3–4	June–July	Dragesund et al. 1980; Runnstrom 1934
27. Plymouth	Stratified	4–5	April–May	Ford 1930; Lebour 1921a
4. Blackwater Estuary	Stratified	4–5	August	Wood 1981
26. Downs	?	6	June	Wood 1959
23. Georges Bank	Well mixed	7	April–May	Boyar et al. 1973b
18. Manx	Well mixed	7	April–May	Cushing 1967
12. Northwestern North Sea (Aberdeen)	Well mixed	7	April–May	Fulton 1906
16. Minch	Well mixed	7	April	De Silva 1973
17. Dogger	Well mixed	7–8	May	Wood 1959
14. Southwest Nova Scotia	Well mixed	7–8	April–May	Das 1968
15. Ile Verte, St. Lawrence Estuary	Well mixed	10–11	Inferred to be during spring	Able 1978 (see text)

blooms. However, studies on Georges Bank have not shown lower phytoplankton biomass within the tidally well-mixed area (Cohen et al. 1982).

If the progeny of "autumn" spawning herring do not "drift" in tidally energetic areas, and only phytoplankton cycles within restricted geographic areas in which the water column is well mixed are evalaluated, no link between timing of spawning and phytoplankton blooms is evident. Quite simply, neither tidally well-mixed water columns nor the transition zones are characterized by autumn blooms. In sum, if the more recent information on phytoplankton seasonal cycles in relation to tidal mixing is considered, and larval retention for herring populations is robust, the hypothesis that herring spawn in relation to phytoplankton blooms is not tenable. In fact, it is difficult to reconcile the wide range in timing of spawning (Fig. 1) with the phytoplankton literature on the timing of predictable blooms.

Timing of Metamorphosis

The literature on the timing of metamorphosis for herring, which we argue is a key element in the definition of the timing of spawning, is not extensive. The available observations suggest that this life history event is constrained to a limited part of the year. For Atlantic herring, metamorphosis is a gradual event, occurring in weeks rather than days and encompassing a broad length range (Ford 1930). Various criteria have been used to assess metamorphosis, including the position of the fins and anus (Ford 1930; Lebour 1921a) and the appearance of scales (Jean 1956; Jensen 1950). Completion of metamorphosis has been reported in herring as small as 30 mm (Wood 1959), while larvae as large as 55 mm have also been recorded (Boyar et al. 1973b). For comparison between populations, a length of 40 mm has been selected to define both the timing of metamorphosis initiation and larval phase duration. Metamorphosis is seasonally restricted and coincides approximately with the productive period of the year (Table 2). The majority of larvae of any one population reach 40 mm during the months April to October inclusive.

In herring, metamorphosis appears to be a physiologically demanding event, accompanied by an increase in haemoglobin synthesis, probably as a result of increased activity (De Silva 1974). Fat levels also increase prior to the life history transition (Marshall et al. 1937). These and related growth processes perhaps explain the seasonal restriction of metamorphosis to the productive period of the year.

The New Hypothesis to Account for the Timing of Spawning of Herring

Two constraints are tentatively identified. First, the larvae of a population develop within the specific oceanographic conditions of its retention area, be it food rich or unproductive, warm or cold, tidally energetic or calm. Second, metamorphosis occurs primarily within a restricted period (April to October). Given these two constraints, it is hypothesized that the timing of spawning of a herring population is a function of the length of time necessary to complete the larval phase and yet metamorphose within the above empirically defined seasonal envelope. Populations that have "good" larval retention areas (be that food rich, or warm, or something else) can spawn in the spring and metamorphose before the autumn. Populations with larval retention areas that are less "good" for larval growth have to spawn earlier (either in the winter or in the fall) to satisfy the two constraints. The differences in larval growth patterns between populations having different larval retention areas are schematically illustrated in Fig. 3. If differences in food availability explain the critical difference in the duration of the larval phase in the respective retention areas, one would expect to observe an inverse relationship between duration of the larval phase for different herring populations and the mean food availability within their respective larval retention areas.

There are two points that need to be addressed in support of this hypothesis: first, that the duration of the larval phase and the larval growth curves vary between the larval retention areas in an appropriate fashion, and second, that zooplankton biomass or production, if it is food availability that defines the "goodness" of a larval distributional area, is inversely related to duration of the larval phase. In support of this hypothesis it is argued that food is an important consideration in determining the growth of the larvae and thus the duration of the larval phase. Temperature as another environmental factor is also briefly considered.

FIG. 3. Generalized depiction of growth rates and timing of metamorphosis for herring larvae spawned in different seasons.

Support for the New Hypothesis

Empirical evidence indicates that the duration of the larval phase for different herring populations varies from 3 to 11 mo (Table 2; Fig. 4). In addition, all herring populations whose larval distributions are coincident with tidally well-mixed waters have relatively long larval phases. The shorter phase durations are associated with populations having larval retention areas in calmer, well-stratified water columns (Table 2). The very rapid and linear growth of the progeny of "spring" spawning populations contrasts with the sigmoid growth curve of "autumn" spawned larvae. The period of no growth in midwinter for several populations suggests that they may be physiologically inactive. These differences in the larval growth characteristics of different herring populations have been well documented since the turn of the century, but they have not been associated with any fundamental differences in the oceanography of the larval distributional areas or accounted for in previous hypotheses concerning the timing of spawning. The "spring" spawning Isle Verte pygmy herring population may be considered the exception that proves the rule (Isle Verte is located in the St. Lawrence Estuary near the shaded area in the inset of Fig. 6). Even though a spring spawner, it is interpreted here that the larval phase of the population is approximately 11 mo long. In October, when growth is slowing, most larvae are 23–28 mm long (Able 1978); thus, it is probable that metamorphosis occurs the following spring. Appropriately, its larval retention area is of the well-mixed, tidally energetic type (Pingree and Griffiths 1980). In sum, the literature on the larval growth patterns of different herring populations supports our hypothesis.

The egg size–fecundity differences observed between herring populations are consistent with the new hypothesis. Herring populations differ considerably in fecundity and egg size (Hempel and Blaxter 1967; Zijlstra 1973). In general, the total energy investment in gonads for a herring of a given size is relatively constant between populations (i.e. varies by a factor of approximately 2.5, while fecundity and egg size vary by factors of 4 and 5, respectively). Thus, there is an inverse relationship between size of eggs and the number produced. There are clear patterns in the fecundity–egg size trade-off: "spring" spawners have relatively fewer eggs of large size and "autumn" spawners have smaller but more eggs. Because of the detailed documentation of the between-population differences in fecundity–egg size, there has been imaginative speculation to explain the observed trade-off by both fisheries biologists and life history strategy ecologists (e.g. see Hempel 1965; Mann and Mills 1979). A simple interpretation consistent with the timing of spawning hypothesis developed here is that, given a "poor" larval retention area and concomitant long larval phase, there will be a greater accumulative population mortality for the larval phase, thus the choice for higher fecundity. The direct relationship between duration of the larval phase and fecundity is shown in Fig. 5. This interpretation does not consider the relatively constant allocation of energy to reproduction among the many herring populations, but does rationalize the subsequent trade-off between egg size and egg number.

Two lines of evidence, incidence of larval feeding and zooplankton biomass, in the various larval retention areas, support the argument that food availability determines the durations of the larval phase. There are, however, difficulties in interpreting the data. Both vertical migration (Fortier and Leggett 1983; Graham 1972; Schnack 1972; Seliverstov 1974; Wood 1971a) and daily "peaks" in feeding activity (Bainbridge and Forsyth 1971; Bhattacharyya 1957; Bjorke 1978; Cohen and Lough 1983) appear to be fundamental in herring larvae. Therefore, observed incidence of feeding (percentage of larvae with food items in their guts) is partially a function of sampling time and depth. There is considerable noise in the data, but some patterns can be identified (Table 3). Populations of spring-spawned larvae have a high percentage of individuals with food in their guts (>70%), whereas those populations having a slower larval growth rate and longer larval phase (in the tidally well-mixed areas) have a lower incidence of feeding.

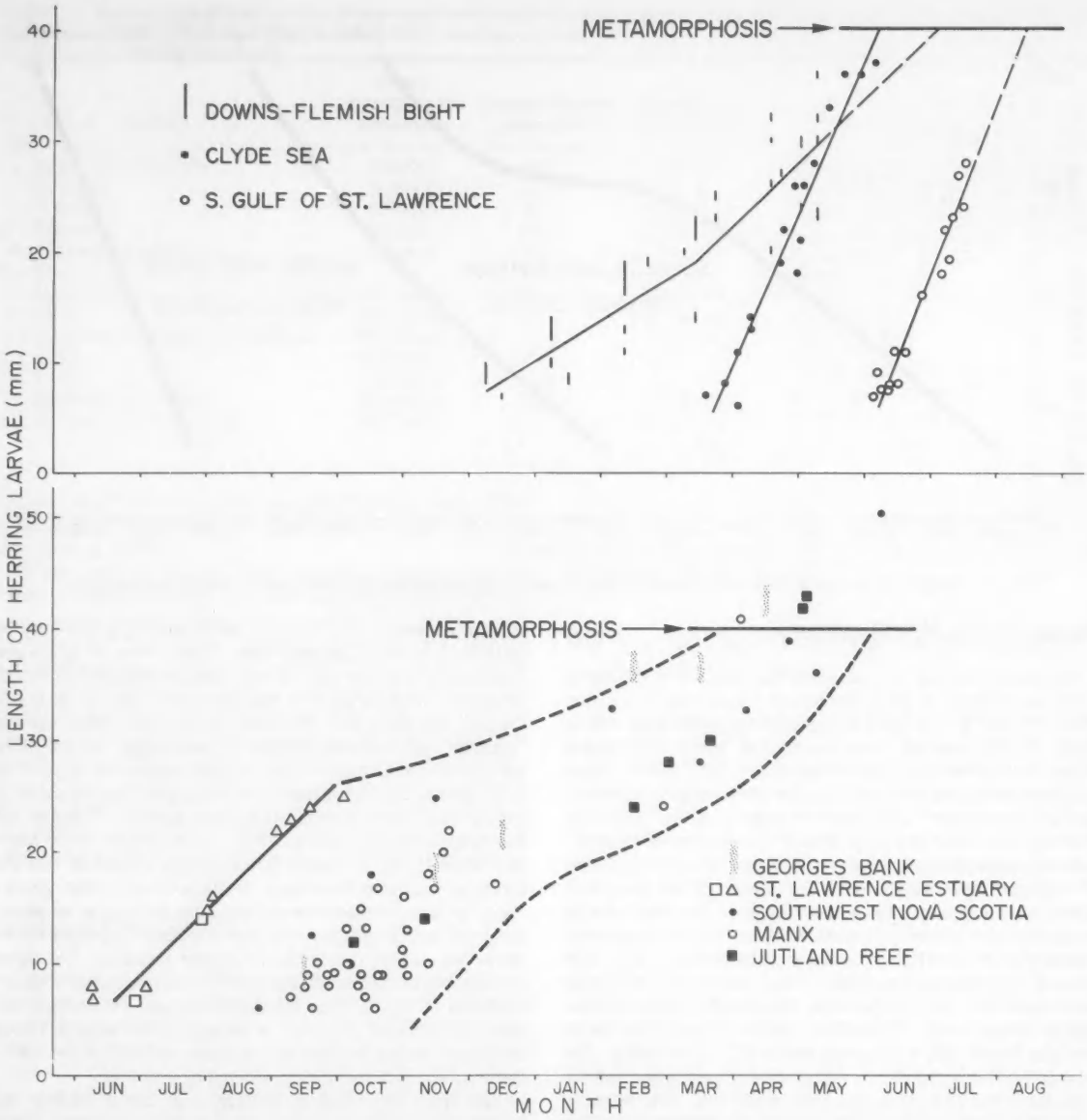

FIG. 4. Growth rates of Atlantic herring larvae from different distributional areas. Points taken from Bückmann (1938) (Downs-Flemish Bight), Marshall et al. (1937) (Clyde Sea), Jean (1956) (southern Gulf of St. Lawrence), Boyar et al. (1973b) (Georges Bank), Able (1978) (triangles), Fortier and Leggett (1982) (squares), Das (1968) (southwest Nova Scotia), Bowers (1952) (Manx), and Jensen (1950) (Jutland Reef). Maximum modal lengths are plotted in most cases. Points from Able (1978) and Jensen (1950) are means; those from Bückmann (1938) are modal lengths from a summary figure. Points from different years were plotted where feasible, e.g. Manx points are from 1949 to 1952.

Zooplankton biomass available to herring larvae in different distributional areas is difficult to compare for several reasons. It is difficult to sample highly aggregated populations. Different investigators use a variety of plankton nets and methods of towing dependent on the specific aims of the studies. The portion of the zooplankton biomass that is utilized by herring larvae is not well known and is assumed here to be related directly to the sampled biomass. These restrictions limit the characterization of larval food availability in diverse areas. Zooplankton displacement volume (Fig. 6) from three larval retention areas representing phase durations of 3, 7, and 11 mo, respectively, nevertheless support the hypothesis. The phytoplankton dynamics shown in Fig. 2 would predict relatively low zooplankton biomass and production within tidally well-mixed areas, and observations support this "prediction" for central Georges Bank and southwest Nova Scotia (Sinclair et al. 1982). Taking Georges Bank as a whole, zooplankton production is not high for a temperate shelf environment being equal to the

TABLE 3. Feeding incidence of larval herring. Only post-yolk sac larvae caught during daylight or during a feeding peak are considered.

Stock	Season	Larval size (mm)	Percent with food	Reference
Schlei Fjord	May	14–20	94–97	Schnack 1972
	June	19–24	74–100	Schnack 1972
Norwegian Sea	April	<20	84	Bjorke 1971
Clyde Sea	March–April (3 yr)	10–20	70 (peak)	Bainbridge and Forsyth 1971
Northwestern North Sea	September	<24	65 (peak)	Bhattacharyya 1957
		14–17	50	Schnack 1972
	October	<25	85 (peak)	Bhattacharyya 1957
	November	<34	60 (peak)	Bhattacharyya 1957
Manx	September	10–15	69	Bowers and Williamson 1951
	October	>10	70	Bowers and Williamson 1951
	November–December	>10	64	Bowers and Williamson 1951
Gulf of Maine	Autumn	7–30	57	Sherman and Honey 1971
	Winter	11–40	51	Sherman and Honey 1971
	Spring (3 yr)	21–50	61	Sherman and Honey 1971
Banks–Dogger	June–November	10–18	50 (peak)	Last 1980
Georges Bank	October (10 yr)	8–12.9	28	Noskov et al. 1978
		13–17.9	33	
	October–November (3 yr)	9–18	21	Cohen and Lough 1983
	December–February (3 yr)	15–28	29	Cohen and Lough 1983
	April–May	34 (mean)	57	Cohen and Lough 1983
St. Lawrence Estuary	June	7–8 (mean)	0–8	R. Courtois and J. J. Dodson,
		10–16 (mean)	23–53	unpubl. data

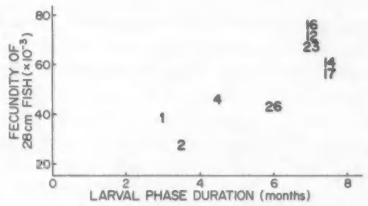

FIG. 5. Length of larval phase versus fecundity for nine Atlantic herring stocks. Stocks corresponding to numbers as in Table 1. Fecundity values from Baxter 1959) (stocks 1, 2, 12, and 26), Wood (1981) (stock 4), Messieh (1976) (stock 14), Perkins and Anthony (1969) (stocks 14 and 23), Wood (1971b) (stock 16), Zijlstra (1973) (stocks 17 and 26), Kandler and Dutt (1958) (stock 17), Schultz (1974) (stock 23), and Burd and Howlett (1974) (stock 26). Means taken where more than one fecundity estimate for a stock available.

Scotian Shelf and two-thirds that of the Gulf of Maine (Cohen et al. 1982). Moreover, transects across Georges Bank indicate that copepod biomass is considerably lower in the central mixed area (Dagg and Turner 1982).

Spring and Autumn Spawning in the Same Area

In several seas that are not tidally energetic, such as the Gulf of St. Lawrence and coastal waters of Iceland, both spring- and autumn-spawning populations spawn in essentially the same location and thus probably share a common larval distributional area. Interestingly, however, not all spawning locations in the Gulf of St. Lawrence are characterized by both spring- and autumn-spawning populations. The Magdalen Islands area sustains only a spring-spawning population, and Pictou Island (located between Nova Scotia and Prince Edward Island in the south part of Magdalen Shallows shown in Fig. 6) only a late summer–autumn one. The relatively rare phenomenon of two populations sharing the same spawning location, and presumably the same larval distributional area, has not been accounted for. An interpretation, albeit speculative, draws on key elements of both the new stock hypothesis (Iles and Sinclair 1982) and the constraints identified above for our timing of spawning hypothesis.

If the physical characteristics of a given geographical area permit larval retention throughout the year, and if there are good growth conditions for herring larvae, then two discrete populations could be sustained using the same larval retention area. One would expect these dual characteristics to be found in only the "calmer" seas, which are characterized by relatively high zooplankton production. Thus, one would not expect to find both spring- and autumn-spawning populations in tidally energetic seas, where the larval distributional areas are coincident with the well-mixed areas of lower zooplankton production. This is the case: tidally energetic seas do not sustain both spring and autumn populations in the same area.

Following this interpretation, areas in the Gulf of St. Lawrence that support two spawning populations should have relatively permanent larval retention characteristics and high summer zooplankton production. The Magdalen Islands larval distributional area, which sustains only a spring-spawning population, may not have a gyral circulation pattern during the autumn–winter period. The Pictou Island spawning location is coincident with a predicted tidally well-mixed area (Pingree and Griffiths 1980), and thus the specific larval distributional area may not be sufficiently "good" to sustain a spring-spawning population. (It is not the aim here to fully explain the timing of spawning question in the Gulf of St. Lawrence, but rather to suggest that the specific characteristics of this area may be interpreted in relation to the new hypothesis.)

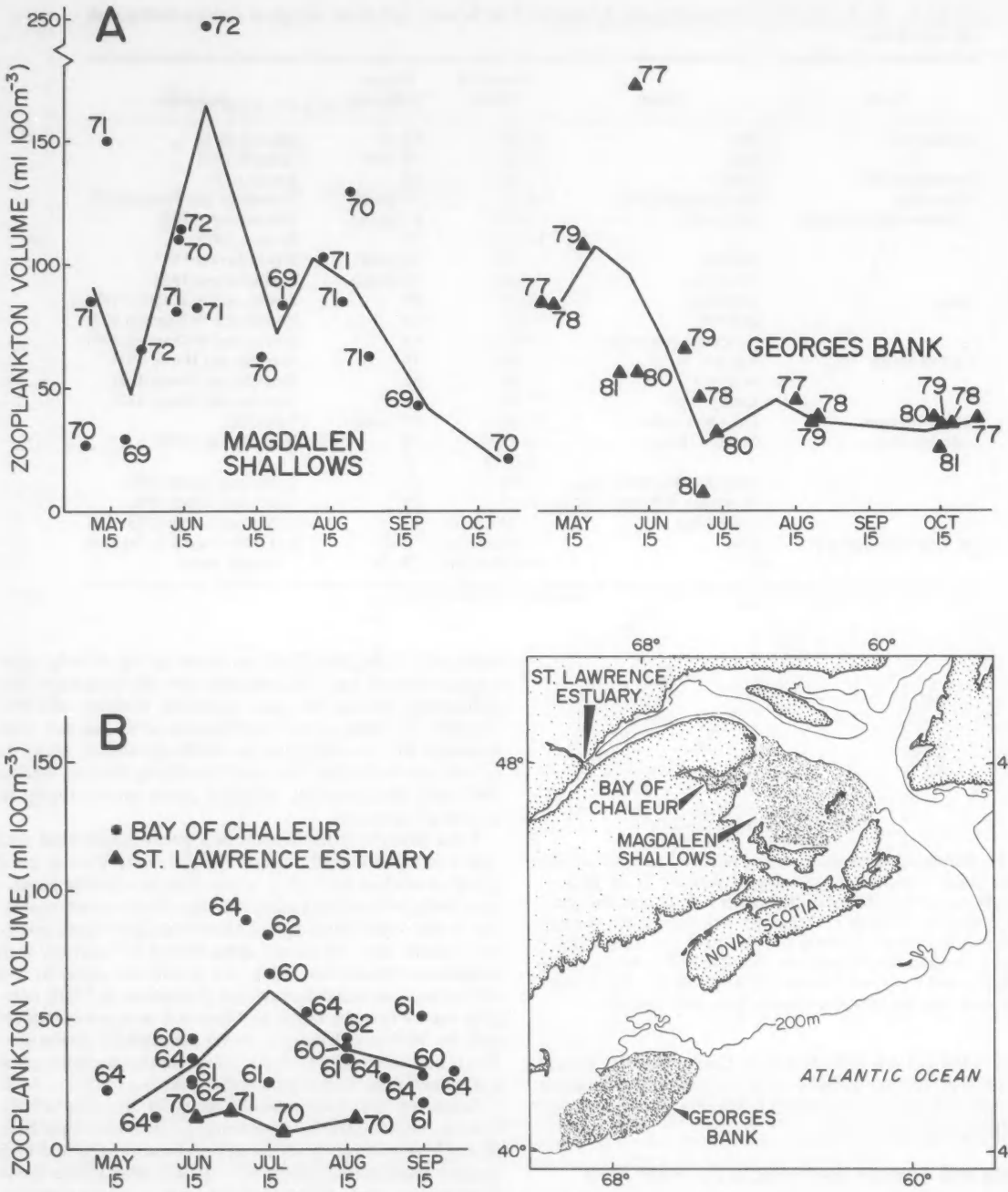

FIG. 6. Comparison of zooplankton volume estimates from different larval distribution areas (location of zooplankton sampling areas indicated by shading on map). (A) Magdalen Shallows versus Georges Bank; (B) Bay of Chaleur versus the St. Lawrence Estuary. Points represent means of 2–52 samples; bold lines indicate mean conditions over years considered. Volumes in Fig. 6A were derived from vertical plankton net tows by Bulleid and Steven (1972), Steven et al. (1973) (Magdalen Shallows, 50-cm net with 230-μm mesh), and Sherman et al. (1982) (Georges Bank, 60-cm net with 333-μm mesh). Volumes in Fig. 6B were derived from Clarke–Bumpus samples by Lacroix and Filteau (1969) (Bay of Chaleur, 336-μm mesh, 0- to 40-m hauls) and R. Coté (Laval University, Ste. Foy, Que., unpubl. data) (St. Lawrence Estuary, 160-μm mesh).

Temperature and Duration of the Larval Phase

Growth of some zooplankton species is independent of food availability (McLaren 1978; McLaren and Corkett 1981) and thus to some degree programmed. Iles (1980) discussed programmed growth in relation to Atlantic herring, and Policansky (1983) has shown experimentally that temperature influences the duration of the larval phase for starry flounder (*Platichthys stellatus*). Temperatures in the surface layers of the different larval retention areas do vary considerably. However, because of vertical migration, larvae in stratified water columns may be spending parts of each day in dramatically different thermal regimes. It is thus difficult to test even qualitatively a relationship between duration of phase and temperature. What specifically defines the goodness of a larval retention area for larval growth is not crucial to the hypothesis. What is central is that differences in oceanographic features between larval retention areas result in differences in the duration of the larval phase. Food availability is developed here as a key factor, but other characteristics of the larval environment may be important.

Conclusions

Timing of spawning of different herring populations is hypothesized to be a function of how "good" the respective larval retention areas are for growth, given the constraint that metamorphosis can only occur within a spring to early autumn seasonal envelope. Even though different populations spawn throughout the year, metamorphosis occurs only during the productive season. In support of the hypothesis is the considerable range in larval phase duration between populations (3–11 mo) and the positive relationship between fecundity and phase length. In addition, zooplankton volume estimates from three larval distributional areas suggest an inverse relationship between phase duration and food available for larval growth. Other environmental factors, or combinations of them, may be important in defining the duration of the larval phase. Two possible factors are temperature and the metabolic energy requirements needed to maintain the discrete larval distributions.

The hypothesis differs from D. H. Cushing's in that timing of spawning is hypothesized to be adapted to the mean characteristics of the specific larval retention area that determine the duration of the larval phase, rather than to seasonal phytoplankton events in the inferred larval drift environments. In addition, timing of metamorphosis is argued to be an important factor determining spawning time. Importantly, timing of spawning and the generation of year-class variability are decoupled in this treatment.

Returning to the steps outlined above in the development of the match–mismatch theory, it can be seen that this decoupling between spawning time and the generation of year-class variance is critical. If spawning of fish is not defined in relation to seasonal blooms, a key component of the match–mismatch theory is removed (the link between spawning events and Sverdrup's critical depth model), and both its rationale and elegance lost.

Acknowledgments

A realization of the importance of the timing of spawning question, as well as many insights into the biology of herring in general, has been provided by our ongoing collaboration with T. D. Iles. His contribution to this paper is gratefully acknowledged. In addition we thank K. Metuzals for translation of several articles in German, J. Dodson, R. Courtois, R. Coté, and K. Sherman for permission to use unpublished data, and R. O. Fournier, R. G. Halliday, R. Mahon, J. McGlade, and C. M. Hawkins for helpful reviews. We appreciate the efficient work done by S. P. Dowell and V. C. Clayton in processing the manuscript, and E. MacDormand in figure drafting.

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Influence of Varying pH and some Inorganic Cations on the Perivitelline Potential of Eggs of Atlantic Salmon (*Salmo salar*)

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Peterson, R. H. 1984. Influence of varying pH and some inorganic cations on the perivitelline potential of eggs of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 41: 1066–1069.

A potential difference is maintained between the perivitelline fluid of the Atlantic salmon (*Salmo salar*) egg and the incubation medium. The magnitude of this potential difference depends on the ionic composition of the incubation medium; it exhibits a maximal negativity in deionized water, then depolarizes, and finally reverses in sign as the ionic content of the incubation medium is raised. The potential difference obeys the Nernst relationship over a limited concentration range of H^+, Ca^{2+}, and Mg^{2+} (10^{-5} to 10^{-3} *M*). The maximal change in potential difference with change in Na^+ and K^+ concentrations is less than that predicted by the Nernst equation. The potential difference permits calculation of the degree of concentration of cations in the perivitelline fluid over that of the ambient medium. Depolarizing the perivitelline potential by lowering the ambient pH will result in losses of other cations from the perivitelline fluid.

Une différence de potentiel est maintenue entre le liquide périvitellin de l'œuf de saumon atlantique (*Salmo salar*) et le médium d'incubation. La valeur de cette différence de potentiel dépend de la composition ionique du médium d'incubation ; elle atteint une négativité maximale dans de l'eau déionisée, puis se dépolarise et finalement change de signe quand la teneur ionique du médium d'incubation est élevée. La différence de potentiel correspond à la relation de Nerst sur un écart limité des concentrations de H^+, de Ca^{2+} et de Mg^{2+} (10^{-5} à 10^{-3} *M*). La variation maximale de la différence de potentiel, quand les concentrations de Na^+ et de K^+ varient, est inférieure à celle prédite par l'équation de Nerst. La différence de potentiel permet le calcul du degré de concentration des cations dans le liquide périvitellin par rapport à celui du médium ambiant. La dépolarisation du potentiel périvitellin par une baisse du pH ambiant entraînera des pertes des autres cations présents dans le liquide périvitellin.

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The acidified precipitation, as a result of industrial emissions, in certain geographic areas, such as eastern North America and Scandinavia, is currently a matter of public concern (Haines 1982). One of the results may be acidification of poorly buffered waters of lakes and streams. Increased H^+ concentration in the waters where teleost eggs are incubated may affect the capacity of the perivitelline fluids to concentrate other inorganic cations, such as Na^+ and Ca^{2+}.

The perivitelline fluid of the teleost egg separates the living tissues of the embryo from the zona radiata. It is formed at water-hardening during the process of imbibition after the release of colloidal material from the cortical alveoli (Bogucki 1930; Gray 1932). This colloid is of sufficient molecular size to prevent its movement through the pores of the zona radiata and is osmotically active, causing water to enter the egg and form the perivitelline fluid.

The perivitelline colloid has a net negative charge at "normal" ambient pH levels and can accumulate cations at concentrations in excess of those of the ambient water (Rudy and Potts 1969). The zona radiata is nonliving and therefore may be presumed to lack the ability to transport ions through metabolically linked processes, with passive diffusion being the sole means of transzonal ion movement. Under these circumstances, concentration differences of diffusible cations across the zona radiata theoretically should be in accordance with a Donnan equilibrium.

One of the characteristics of a Donnan equilibrium is the establishment of a potential difference between the solutions on either side of the diffusion barrier; this potential henceforth is termed the perivitelline potential, or "pvp". The pvp theoretically is related to the cation concentration differences across the zona radiata by the Nernst equation:

$$\text{pvp} = -\frac{RT}{nF} \ln \frac{C_i}{C_o}$$

where R is the gas constant, T is the absolute temperature, n is the valence of the ion, F is the Faraday constant, and C_i and C_o are the concentrations of the ion inside and outside the zona radiata, respectively. If one converts to Briggsian logarithms and combines constants, the pvp may be related to the concentration difference by a slope of 56.5 mV at 12°C, the perivitelline fluid being negative with respect to the ambient water. Where more than one ion is involved, the more general Goldman equation

$$\text{pvp} = -\frac{RT}{nF} \ln \frac{r_1[X_1]_i}{r_1[X_1]_o} + \frac{r_2[X_2]_i}{r_2[X_2]_o} + \ldots + \frac{r_n[X_n]_i}{r_n[X_n]_o}$$

may be applicable, where $(X_1, \ldots, X_n)$ are concentrations of various ions and $(r_1, \ldots, r_n)$ are the respective permeabilities.

The only published measurement of the pvp is apparently that of Sumwalt (1929) who performed pvp measurements on *Fundulus* (probably *heteroclitus*) eggs. She obtained maximum

slope functions of 47.1 mv/decade change in K^+ for concentrations of 5×10^{-3} to 5×10^{-4} *M* (*M* = mol/L), considering this to approximate the Nernst relationship. Sumwalt interpreted her results by using the dried collodian membrane as a model. This model was applied incorrectly, as potential differences obtained across the collodian membrane were believed to be due to the impermeability of inorganic anions such as Cl^- rather than that of large negatively charged, nondiffusible protein molecules. Both models, however, theoretically should yield a Nernst relationship for variable cation concentration differences. Rudy and Potts (1969) concluded that a pvp did exist, reasoning from a Donnan relationship and the higher Na^+ content of perivitelline fluid under certain conditions, but did not measure the pvp. The pvp, in simple terms, may be regarded as a measure of the capacity of the perivitelline fluid to concentrate cations above the concentration present in the ambient water. In this study, the relationships between the pvp and concentrations of H^+, Na^+, K^+, Ca^{2+}, and Mg^{2+} in the ambient water were determined.

Materials and Methods

Atlantic salmon (*Salmo salar*) eggs from stock of Big Salmon River, N.B., were collected and fertilized at the North American Salmon Research Center in Chamcook, N.B. They were incubated in conditions described in Peterson and Martin-Robichaud (1983).

All potential measurements were performed on eggs subsequent to formation of eye pigmentation. Experiments terminated approximately a week before hatching. The electrodes more readily penetrated eggs near hatching, probably because of partial enzymic degradation of the zona radiata; however, potentials measured under standard conditions (i.e. St. Andrews tapwater at 12.5°C) did not change as development proceeded, indicating that colloid impermeability was maintained throughout the period of measurement. All electrode penetrations were performed on eggs with the use of Narishige® micromanipulators. The eggs were illuminated from the side and viewed at 125× with a stero microscope. About one half of the egg was immersed in a bath of the desired ionic composition, and the electrode penetration was at the apex of the exposed half. Only penetrations that clearly made no contact with the embryo or vitelline membrane were used. Periodically, eggs were immersed completely in the bath after the potential was recorded to see if potential changes occurred because of short circuits around the site of penetration. No such short circuit was observed. Usually the zona radiata became sealed to the electrode after penetration; eggs remained attached if raised from the bath on the electrode and normally required a slight tap with forceps for removal. Each egg was penetrated only once, although the puncture appeared to seal after electrode removal, and embryos in penetrated eggs showed no subsequent ill effects.

The potentials were recorded with drawn-glass micropipets with a 10 to 30-μm tip size. A strand of glass fiber was inserted in each glass blank, to serve as a wick, prior to drawing on a Narishige® vertical electrode puller. The drawn electrodes were filled with 4 *M* NaCl dissolved in 1% agar to eliminate bulk flow of electrolyte solution into the pervitelline fluid. Potentials measured with the electrodes did not differ from those measured with electrodes filled with 3 *M* KCl–1% agar, so that the potentials cannot be ascribed to tip potentials created by differing ionic mobilities within the microelectrode. The NaCl-filled electrodes were used for all measurements given here, as Na^+ is probably the principal inorganic cation in the perivitelline fluid. It is also unlikely that the potentials measured were due to concentration differences between the perivitelline fluid and tapwater or distilled water, as potential differences between distilled water and 0.004–0.4 *M* NaCl were usually only 0.5–2 mV in magnitude (as measured in the system given below where || represents the contact zone between different electrolytes and | represents an electrode–solution interface):

(in 1% agar bridge)

Ag:AgCl|4 *M* NaCl||Dist. H_2O||Sat. KCl||0.4 *M* NaCl||4 *M* NaCl|Ag:$AgCl_2$

(microelectrode) (microelectrode)

Potentials were led directly to a Keithley® model 602 electrometer with 100 TΩ impedance shorted by 20 pf. The low impedance input was shorted to ground. The potential was recorded as the difference between two microelectrodes, one with the tip immersed in the solution bathing the egg connected to the low impedance lead and the other with tip immersed in the perivitelline fluid and connected to the high impedance input. Before each egg was penetrated, the two microelectrodes were immersed in the bathing solution and the electrometer reading adjusted to zero. After each measure of pvp, the recording electrode was again dipped into the bathing solution and any potential existing due to electrometer drift or electrode contamination was noted (usually 0 to ±2 mV). This potential was used to correct the pvp recorded. All values of pvp given in the results are means of measurements on 10 eggs. All solutions, unless stated otherwise, were made up with distilled deionized water with a resistivity greater than 10 MΩcm. The pH of test solutions was measured after pvp measurement to ensure that K^+ leakage from pH electrodes did not affect potential measurements.

Results

The pH of the ambient medium affects the measured pvp (Fig. 1). The change in pvp, as $[H^+]$ is varied, conforms very well to the predictions of the Nernst relationship of 56.5 mV/decade change in H^+ (given by broken line) over a concentration range of 10^{-5} to 5×10^{-4} *M*. At higher and lower concentrations the slope decreases and then tends to approach zero. At approximately 3×10^{-4} *M* H^+, the pvp reverses to a positive potential that levels off at about +50 mV for $[H^+]$ of 10^{-2} *M*. At H^+ concentrations less than 10^{-5} *M*, the pvp becomes constant at about −80 mV. I have obtained similar results by using HCl rather than H_2SO_4.

The pvp–K^+ relationship (Fig. 2) differs from the results obtained with H^+ in that the maximal slope is somewhat less than the Nernstian slope, about 40 mV/decade change in K^+ concentration (at pH 5.8), and the potential at high concentrations stabilizes at about +25 mV as opposed to +50 mV for H^+.

The response to Na^+ has a lower slope than that for the response to K^+ (Fig. 3). The maximum slope at pH 5.8 is

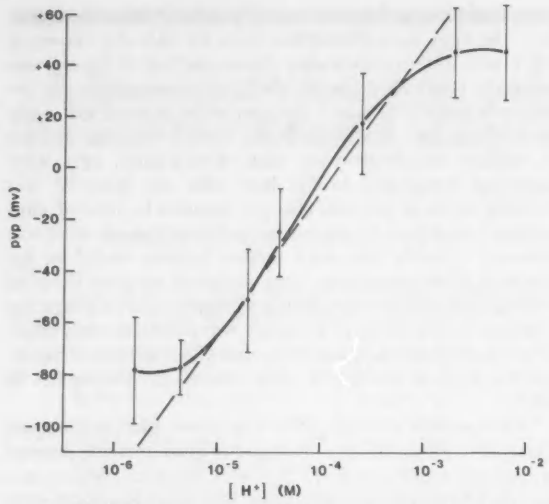

FIG. 1. Potential difference (pvp) between the perivitelline fluid and the incubation medium as a function of ambient H^+ concentration. The eggs were incubated in deionized water acidified with H_2SO_4. Each point is the mean of 10 measurements; bars indicate ±1 SD. The broken line indicates the slope predicted from a Nernst relationship.

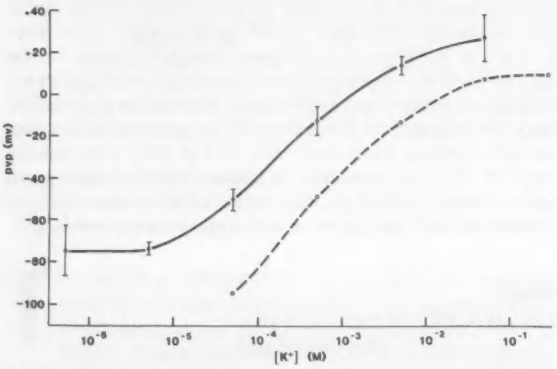

FIG. 2. The pvp for various K^+ concentrations (as KCl) in deionized water (solid line). The broken line is the relationship obtained by Sumwalt (1929) for *Fundulus* sp. eggs. Bars indicate ±1 SD.

30 mV/decade change in Na^+, and the potential does not reverse at high concentrations, stabilizing near 0. If the pH of the ambient medium is reduced to 4.2, then the pvp is less responsive to Na^+ change at concentrations less than 10^{-3} *M*, with the potential stabilizing at −45 mV at low concentrations compared with −70 mV at pH 5.8. At Na^+ concentrations of 10^{-3} *M* and greater, the response is similar for both pH levels.

For comparative purposes, some values of pvp have been calculated from the presumed Donnan relationship (triangles in Fig. 3) using values of Na^+ measured by Rudy and Potts (1969) for perivitelline fluid and ambient water for Lune River salmon. These calculated pvp values are nearly identical to those measured in my experiments for ambient concentrations of 10^{-4}, 10^{-2}, or 10^{-1} *M*, with the calculated value at 10^{-3} *M* Na being slightly lower than the potentials measured in my experiments.

The response of the salmon egg pvp to changes in $[Ca^{2+}]$ and $[Mg^{2+}]$ follows a Nernstian slope for a concentration range of

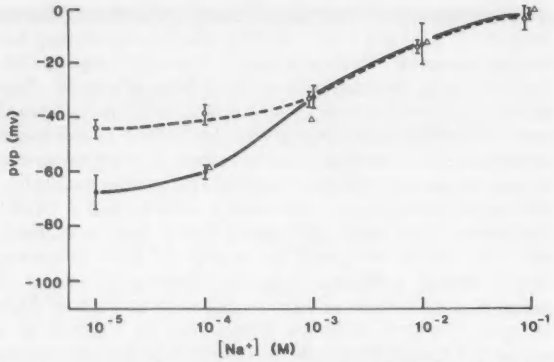

FIG. 3. The pvp for various concentrations of Na^+ (as NaCl) in deionized water. The solid line indicates the relationship at pH 5.7, the broken line at pH 4.2. Bars indicate ±1 SD. The triangles are calculated values of pvp based on Na^+ concentration differences published by Rudy and Potts (1969), assuming a Donnan equilibrium.

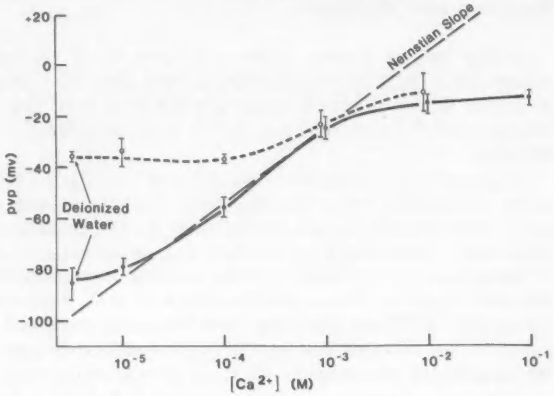

FIG. 4. Solid line: pvp for various Ca^{2+} concentrations (as $CaCl_2$) in deionized water at pH 5.7. Broken line: as for solid line, but at pH 4.1. Bars indicate ±1 SD.

10^{-5} to 10^{-3} *M* (Fig. 4 and 5, respectively), the slope for a divalent ion being 28 mV/decade change, half that for a univalent ion. The pvp does not become positive at high divalent cation concentrations but stabilizes at about −10 mV. As was the case with Na^+, the response to Ca^{2+} in deionized water acidified to pH 4.1 was depressed at $[Ca^{2+}]$ less than 10^{-3} *M* (Fig. 4), with the pvp becoming stabilized at about −38 mV.

The pvp of salmon eggs bathed in the normal laboratory tapwater (pH = 6.8, Na^+ = 0.1–0.2 m*M*, $Ca^{2+} \approx 50\ \mu M$, $Mg^{2+} \approx 24\ \mu M$) was measured. The mean pvp was −44 mV (SD ± 8.4, n = 21). For eggs that had been incubated in tapwater adjusted to pH 4.5 after becoming water-hardened, the pvp was −5.2 mV (SD ± 6.6, n = 6).

Discussion

In deionized water, the pvp of the salmon egg becomes an ideal hydrogen electrode at H^+ concentrations ranging from 3×10^{-5} *M* to 4×10^{-4} *M* (pH range of 4.5–3.4) with a slope identical to the calculated change of 56.5 mV/decade. Beyond this range the response decreases and the slope becomes zero at concentrations greater than 10^{-3} *M* or less than 10^{-5} *M*. The decreased response at low $[H^+]$ (higher pH) may be the result of

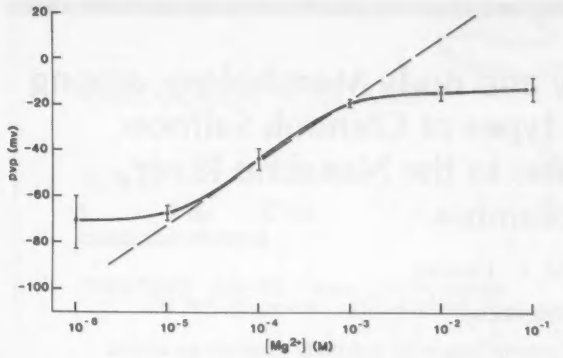

FIG. 5. The pvp for various concentrations of Mg^{2+} (as $MgCl_2$) in deionized water. The broken line indicates the theoretical slope predicted by the Nernst equation. Vertical bars indicate ±1 SD.

two factors. First, as stated by Rudy and Potts (1969), Donnan ratios are not maintained in very dilute media because of association of cations, which are concentrated by the pvp, with the anionic radicals on the colloid. The colloidal anionic excess is thus reduced. Second, at these low H^+ concentrations, there may be sufficient residual ions remaining in the "deionized" water to interfere with the response to the hydrogen ion. A conductivity of 10^{-7} μmho/cm (= resistivity of 10 MΩcm) would correspond to an ionic equivalent of about 10^{-6} *M*.

The pvp becomes zero at about pH 4.0, indicating that there is no net charge on the perivitelline colloids at this pH. At greater H^+ concentrations, the pvp reverses to a positive potential, indicating a tendency to concentrate anions. This is probably due to the amphoteric nature of some of the radicals on the perivitelline colloids, so that a net positive charge occurs at very low pH levels. At pH 2, the concentrations of H^+ probably approaches that of the fixed charges on the colloids, so that the system is saturated with H^+ and the pvp is stabilized at about +45 mV. Rudy and Potts (1969) estimated the fixed charge at about 10 mequiv/kg. The capacity of the perivitelline fluid to concentrate cations is reduced as the pH is lowered below 5.5 and is lost at a pH of 4.0 or less. The presence of other cations in the ambient medium in addition to H^+ would tend, however, to diminish the response to pH change, particularly at higher pH levels.

The interference between two species of cations is illustrated in the responses to change in ambient Na^+ at two different pH levels (Fig. 3) where the response to Na^+ at pH 4.2 is decreased for Na^+ concentration less than 10^{-3} *M*. This situation is analogous to the mixed response of a selective ion electrode to two ion species (Covington 1979), and a selectivity coefficient may be calculated from $K_{Na,H} = C_{Na(H)}/C_H$ where $K_{Na,H}$ is the selectivity coefficient, $C_{Na(H)}$ is the Na concentration at the midpoint of the "mixed" response (obtained by extrapolation of the horizontal part of the pH 4.0 curve to the pH 5.8 curve and reading the Na^+ at that point), and C_H is the H^+ concentration (10^{-4} *M*). A selectivity coefficient of $(4 \times 10^{-4})/(5 \times 10^{-5}) = 8$ is obtained. In other words, the pvp is about 8 times as sensitive to H^+ as to Na^+. Similarly, from Fig. 4, the calculated pvp is some 50 000 times as sensitive to H^+ as to Ca^{2+}. The value is much higher because the denominator is squared in the case of interference with divalent cations.

The slope of the pvp response to change in ambient cation concentration is greatest for H^+, lower for K^+, and still lower for Na^+, being 56, 40, and 30 mV/decade change, respectively. This difference in response may be caused by differing mobilities of these ions in aqueous solution, resulting in varying permeabilities through the zona radiata. The mobility of hydrated hydrogen ion is 32.7×10^{-4} cm$^2 \cdot$V$^{-1} \cdot$s^{-1}, while those of K^+ and Na^+ are 6.7×10^{-4} and 4.5×10^{-4} cm$^2 \cdot$V$^{-1} \cdot$s^{-1}, respectively. The ratios of the mobilities of H^+ and Na^+ (approximately 7–8) also agree well with the selectivity coefficient, about 8, calculated for these two ions. The extremely large selectivity coefficient calculated for Ca^{2+} relative to H^+ implies that some factor other than ion mobility is responsible. Adsorption of Ca^{2+} to sites on the zona radiata may decrease permeability of this ion through the zona radiata. Adsorption of divalent ions, such as Cd^{2+}, to the zona radiata has been documented (Beattie and Pascoe 1978).

Reasons for the pvp of *Fundulus* eggs to be 20–40 mV more negative at a given K^+ concentration than those for the salmon pvp recorded in my experiments (Fig. 2) are conjectural. One possibility is that the concentration of perivitelline colloid was higher in the *Fundulus* egg. This would be reasonable for a marine species, since more colloid might be required to generate an osmotic pressure differential across the zona radiata to allow hardening to occur.

In experiments with deionized water, a maximum pvp of about −80 mV was obtained (at pH 5.8). The pvp of salmon eggs in normal tapwater was about −45 mV. Since tapwater contains about 10^{-4} *M* Na^+ and 5×10^{-5} *M* Ca^{2+}, the concentrations of these two ions could largely account for the differences in pvp obtained in the two media.

Whether the ability of the perivitelline fluids to concentrate cations at pH levels down to about 4.0 confers any advantage to salmon eggs developing in acid water is conjectural. Newly hatched alevins were more sensitive to low pH than unhatched embryos (Daye and Garside 1977). Whether some of the increased tolerance prior to hatching can be attributed to the composition of the perivitelline fluid awaits further experimentation.

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Differences in Enzyme Frequency and Body Morphology among Three Juvenile Life History Types of Chinook Salmon (*Oncorhynchus tshawytscha*) in the Nanaimo River, British Columbia

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Chinook salmon (*Oncorhynchus tshawytscha*), in the Nanaimo River and elsewhere, exhibit three juvenile life history types characterized by different ages at seaward migration. One type migrates to sea immediately after emergence from the spawning gravel and rears in high-salinity estuarine habitats, a second migrates seaward after rearing for about 2 mo in freshwater, and a third type after rearing for a year in freshwater. Nanaimo River chinook were polymorphic at 10 of 31 loci examined electrophoretically. The frequency of allozymes differed significantly among the three life history types at 4 of the 10 loci. A significant deficiency of heterozygotes at the locus for PMI-2 characterized juveniles that reared in estuarine habitats. Fry that died on transfer to salt water in the laboratory had a significant excess of PMI-2 heterozygotes, suggesting that this locus may be associated with salinity adaptation. The three life history types also differed significantly in body morphology. Fish that reared in the estuary had slimmer bodies, smaller heads, and shorter fins than those that reared in the river. Those that spent a year in freshwater had the largest heads, deepest bodies, and longest fins. These observations corroborate the hypothesis that the three life history types represent genetically isolated subpopulations that appear to be physically adapted to their rearing environment. Current plans to increase the numbers of chinook available to commercial and recreational fishermen through artificial propagation of chinook must be made compatible with this degree of genetic variation.

Chez le saumon quinnat (*Oncorhynchus tshawytscha*) de la rivière Nanaïmo et d'ailleurs, il existe trois types de stade juvénile caractérisés par des âges différents à la migration vers la mer. L'un des types migre immédiatement après l'émergence des frayères natales et effectue sa croissance dans des habitats estuariens à salinité élevée ; un deuxième migre après environ 2 mo de croissance en eau douce tandis que le troisième se déplace vers la mer après 1 an en eau douce. Une analyse électrophorétique a révélé, chez le quinnat de la Nanaïmo, l'existence de polymorphes à 10 des 31 loci étudiés. La fréquence des allozymes variait de façon significative entre les trois stades juvéniles à 4 des 10 loci. Les juvéniles des eaux estuariennes étaient caractérisés par une déficience significative d'hétérozygotes au locus PMI-2. Les alevins morts après le transfert en eau salée, au laboratoire, possédaient un excès significatif d'hétérozygotes PMI-2, ce qui porte à croire que ce locus peut être associé avec une adaptation à la salinité. La morphologie corporelle associée aux trois stades variait aussi de façon significative. Ainsi, les quinnats vivant en estuaire avaient des corps plus élancés, des têtes plus petites et des nageoires plus courtes que ceux des eaux douces. Ceux qui ont passé une année en eau douce avaient les plus grosses têtes, les hauteurs du corps les plus élevées et les plus longues nageoires. Ces observations viennent corroborer l'hypothèse selon laquelle les trois différents stades juvéniles s'appliquent à des sous-populations génétiquement isolées qui semblent être physiquement adaptées à leur environnement d'élevage. Les plans actuels visant l'augmentation du nombre de quinnats disponibles pour la pêche commerciale et sportive, par l'entremise de la propagation artificielle, doivent être compatibles avec ce degré de variation génétique.

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Numerous studies have shown that chinook salmon (*Oncorhynchus tshawytscha*) exhibit three different juvenile life history patterns distinguished by the age at which the fish move to the ocean. One type (subyearling estuarine smolts) moves as fry into the river estuary shortly after emergence from the spawning gravel and rears to smoltification there, dispersing seaward in June and July (Healey 1980, 1982; Levy and Northcote 1982). A second type (subyearling riverine smolts) rears within the river for about 2 mo following emergence and then smoltifies and migrates to the estuary. These fish remain in the estuary for a short time before entering the ocean proper (Rich 1920; Reimers 1973; Healey 1982). A third type (yearling riverine smolts) spends a year in the river, smoltifying and migrating seaward in the spring following their emergence (Rich 1920; Healey 1982).

Rich (1920) argued that age of seaward migration was a

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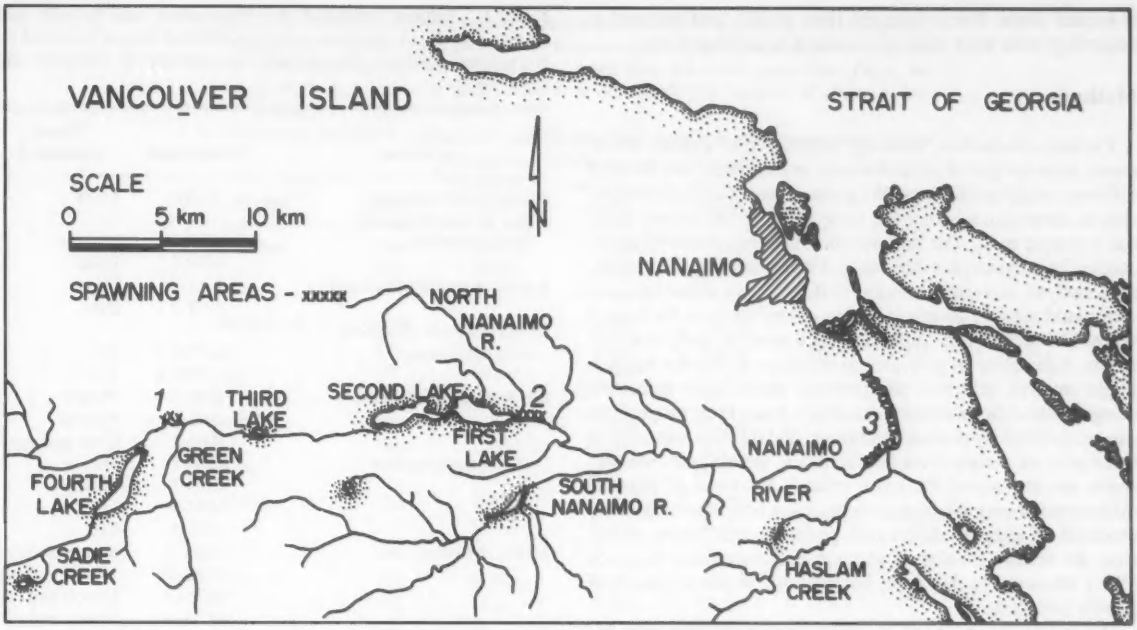

FIG. 1. Nanaimo River watershed showing the location of the landmarks referred to in the text and of the upper (1), middle (2), and lower (3) spawning areas.

phenotypic characteristic dependent on the "harshness" of the rearing environment. Rich and Holmes (1928), on the other hand, presented evidence that the age of seaward migration was inherited. The genetic contribution to life history variation in Pacific salmon is not well documented, although there is good reason to believe that it is significant (Raleigh 1967; Calaprice 1969; Ricker 1972; Healey 1983). Fishery managers and biologists are concerned over the potential loss of genetic variation in economically valuable species due to selective harvesting and artificial propagation and are uncertain about the effects of this loss on stock productivity (Larkin 1981; Ricker 1981; MacLean and Evans 1981). If we are to address this concern, it is essential that we increase our understanding of the genetic and environmental contributions to life history variation in salmon. Because of their variable life history (Healey 1982, 1983), chinook salmon are an ideal subject for such an investigation.

The three juvenile life history types of chinook we have described are documented for the Nanaimo River, British Columbia, and are believed to originate, at least in part, from subpopulations that spawn in separate areas of the river (Healey 1980, 1982, and unpubl. data; Healey and Jordan 1982). Our objective was to determine whether juveniles migrating seaward at different ages also differed in other ways that would indicate genetic control of age of seaward migration. Specifically, we compared the frequency of allozymes of polymorphic enzymes and measurements of certain body dimensions among the three juvenile life history types.

Study Area and Background

The Nanaimo River is a sixth-order stream (Vannote et al. 1980) on the east coast of Vancouver Island just south of the city of Nanaimo (Fig. 1). The river is approximately 56 km long and has a watershed of 830 km^2. The system includes four small lakes and two storage reservoirs. Flow varies from about 3 m^3/s in the summer to over 400 m^3/s during winter freshetts and averages about 42.5 m^3/s.

Since 1950, chinook salmon escapement to the Nanaimo River has averaged about 2100 spawners. Chinook spawn in three general regions of the Nanaimo River, an upper, a middle, and a lower region (Fig. 1; 1, 2, and 3). Adults that spawn in the middle (1191 in 1980) and upper (65 in 1980) regions enter the river between February and June and spawn during the first part of October. Adults that spawn in the lower region (2136 in 1980) enter the river in late September and spawn about the same time as, or slightly later than, those in the middle and upper regions. Scales of adults collected in 1980 and 1981 indicated that 50% of the spawners in the upper region but only 8% from the middle region and less than 1% from the lower region had spent a year in freshwater before going to sea (Healey and Jordan 1982).

Fry from both middle and lower spawning regions drift down to the estuary to become subyearling estuarine smolts. Subyearling riverine smolts generally rear downstream from the middle spawning region although some occur in the lower reaches of the river. Yearling riverine smolts rear downstream from the upper spawning region. These fish spend the winter in First and Second lakes (Fig. 1) and possibly also in deep river pools. Thus, although there is some physical separation of adult spawning groups that produce the juveniles showing different migratory behavior, the separation is not complete.

In 1979, the Nanaimo River hatchery began releasing juvenile chinook into the river, the offspring of adults taken from the lower and middle spawning regions. The offspring from each region were reared separately in the hatchery, which

is located about 10 km from the river mouth, and released as fingerlings near their respective natural spawning areas.

Methods

Current information, although suggestive of genetic differences, does not permit us to discount an environmental cause of different migratory timings. We gathered two kinds of information to determine whether the three juvenile life history types had a genetic basis. The first was the frequency of alleles at loci coding for polymorphic enzymes, as determined by electrophoretic analysis of various tissues. Differences in allelic frequencies would indicate genetic isolation among the three life history types. The second was comparison of a series of body measurements. Adaptation of juveniles to estuarine or riverine habitats might involve selection for physical differences detectable using standard morphometric measures. Since body proportions can be modified by rearing conditions, and the data presented in this report are mainly from field samples, the enzyme measurements are considered the more critical. Evidence of physical differences among life history types is an important adjunct to evidence of enzyme differences given the continuing debate over the selective value of enzyme polymorphisms (Clayton 1981) although, in this case, not essential to the argument of genetic isolation.

We collected samples of juveniles representing the three life history types between 1980 and 1982. We collected subyearling estuarine smolts on the Nanaimo Estuary from March to early May by beach seine, 107 being collected in 1981 and 128 in 1982. We captured subyearling riverine smolts during their seaward migration in late May and early June by inclined plane trap and by fyke net, 63 being collected in 1981 and 30 in 1982. We captured 44 yearling riverine smolts between August 1980 and May 1981 by small-mesh gill net in First and Second lakes (Fig. 1) by fyke net above the lakes and by inclined plane trap in the river, 10 being collected in 1980 and 34 in 1981. The separate samples permitted comparison of allelic frequencies between year-classes for each life history type.

We captured additional samples of river rearing juveniles, believed to be virtually all underyearling riverine smolts, but possibly including one or two that would become yearling smolts, by dip net and beach seine in the middle spawning region of the river in April and May of 1981 and 1982. We captured 218 in 1981 and 84 in 1982.

We placed all field samples on ice at the time of capture and, within 3 h of capture, stored them at −80°C in the laboratory.

The field samples included fish that varied in age from a few weeks to over a year and that had experienced different rearing conditions. In particular, the fish from the estuary had to make the transition to salt water (Healey 1980), while those from the river did not. Comparisons among the field samples could, therefore, be confounded by ontogenetic or environmentally induced changes in enzyme expression and morphometry. To determine whether such changes occurred, we obtained two groups of recently emerged fry from the Nanaimo River hatchery and reared them in aquaria at the Pacific Biological Station under natural photoperiod length and ambient water temperatures. One of the groups of fry was the progeny of approximately 30 adults from the lower spawning region and thus represented subyearling estuarine smolts. The second group was the progeny of approximately 30 adults from the middle spawning region and thus represented subyearling riverine smolts and possibly also some subyearling estuarine smolts. We divided each group between two 60-L freshwater tanks (a total of four tanks), placing approximately 300 fish in each tank. After acclimatization to the laboratory, we changed one tank from each group to salt water (approximately 32‰) over a 7-d period (April 15–21, 1981). We fed all groups on freeze-dried euphausiids and Oregon Moist Pellets (Oreaqua Inc.). Water temperature in all tanks increased from 7 to 10°C during the experiment.

We recorded and froze all mortalities. We took samples for electrophoretic and morphometric measurements from each tank on April 10, 5 d before the transfer of two tanks to salt water, and on May 31, 40 d after full acclimatization of the fish transferred to salt water.

We performed horizontal starch–gel electrophoresis on liver, eye, and muscle tissues, as described by Utter et al. (1974). We used two buffer systems (Ridgway et al. 1970; Clayton and Tretiak 1972) and stained the gels as described by Allendorf et al. (1977). We routinely scored 16 enzymes, encoded by 31 loci (Table 1).

We measured wet weight, fork length, head and body depth, head and snout length, pectoral fin length, and eye diameter, as described by Hubbs and Lagler (1958), on thawed fish, most of

TABLE 1. Enzymes analyzed, the abbreviations used for each, and the tissue analyzed. Isozymes coded by different loci are indicated by postscripted numbers. Nomenclature is according to Allendorf and Utter (1979).

Enzyme	Abbreviation	Tissue examined
Alcohol dehydrogenase	ADH	Liver
Alpha glycerophosphate dehydrogenase	AGP-1	Muscle
	AGP-2	Liver
Aspartate amino transferase	AAT-2	Liver
	AAT-3	Eye
Glyceraldehyde phosphate dehydrogenase	GAPDH-1	Eye
	GAPDH-2	Eye
Isocitrate dehydrogenase	IDH-1	Muscle
	IDH-2	Muscle
	IDH-3	Liver and eye
Lactate dehydrogenase	LDH-1	Muscle
	LDH-3	Muscle
	LDH-4	Liver
	LDH-5	Eye
Malate dehydrogenase	MDH-1	Muscle and eye
	MDH-2	Muscle and eye
	MDH-3	Muscle and eye
	MDH-4	Muscle and eye
Malic enzyme	ME-1	Muscle
Peptidase-2 (substrate, glycyl leucine)	GL-2	Muscle and eye
Peptidase-3 (substrate, leucyl glycylglycine)	LGG	Muscle and eye
6-Phosphogluconic dehydrogenase	6-PGD	Muscle
Phosphoglucose isomerase	PGI-1	Muscle
	PGI-2	Muscle
	PGI-3	Muscle
Phosphoglucomutase	PGM-1	Muscle
	PGM-2	Liver
Phosphomannose isomerase	PMI-2	Liver and eye
Sorbitol dehydrogenase	SDH-1	Liver
	SDH-2	Liver
Superoxide dismutase	SOD	Liver and eye

TABLE 2. Allelic frequencies at loci coding for polymorphic enzymes in wild subyearling estuarine and riverine smolts and yearling riverine smolts. χ^2 and probability values are for the hypothesis that the wild samples are drawn from the same population. Fst is the weighted fixation index for each locus. N = number of fish; number of alleles is $2N$. $^*P < 0.05$; $^{**}P < 0.01$.

Locus	Allele	Subyearling estuarine smolt	Subyearling riverine smolt	Yearling riverine smolt	χ^2	Fst
MDH-1	100	0.998	1.000	1.000	1.87	0.000
	120	0.002	0.000	0.000		
N		235	395	44		
MDH-3,4	100	0.974	0.995	1.000	12.98**	0.000
	120	0.022	0.003	0.000		
	80	0.004	0.002	0.000		
N		235	395	44		
6-PGD	100	1.000	1.000	0.989	14.33**	0.048
	90	0.000	0.000	0.011		
N		235	395	44		
LGG	100	0.690	0.676	0.833	0.73	0.000
	115	0.310	0.324	0.167		
N		63	145	3		
AAT-3	100	0.783	0.880	0.902	19.72**	0.005
	90	0.217	0.120	0.098		
N		226	267	41		
IDH-2	100	0.996	0.997	1.000	0.56	0.001
	120	0.004	0.003	0.000		
N		235	390	44		
PMI-2	100	0.513	0.538	0.457	1.96	0.002
	88	0.487	0.462	0.543		
N		231	317	35		
SDH-1	100	0.853	0.913	0.865	9.04*	0.004
	10	0.147	0.087	0.135		
N		194	311	26		
SOD	100	0.990	0.993	1.000	0.69	0.002
	60	0.010	0.007	0.000		
N		145	218	25		
LDH-5	100	0.994	0.993	1.000	0.50	0.002
	95	0.006	0.007	0.000		
N		235	383	38		

which were subsequently used for enzyme analysis. Not all fish used for enzyme analysis were measured because of time constraints that would jeopardize the more important enzyme analysis.

We compared the frequency of genotypes for enzymes that were polymorphic with Hardy–Weinberg equilibrium, and allele frequencies among samples, by Chi Square contingency tables using the Yates correction for small sample size. We calculated fixation indices (Fst) (Hartl 1980) for polymorphic loci and genetic distance between reproductively isolated groups (Nei 1972).

We compared body measurements among life history types by analysis of covariance of the $\log_e$ transformed measurements. The $\log_e$ transformation was used to normalize distributions and stabilize variances. Fork length was the covariate in all comparisons and we compared both treatment means adjusted to a common fork length and regression coefficients of the various measures regressed on fork length for each life history type.

Results

Hatchery fry reared in the laboratory and sampled on April 10 and May 31 were analyzed for 6 of the 10 polymorphic loci found in the general population (MDH-3,4, IDH-2, PMI-2, SDH-2, SOD, LDH-5). Treating offspring from different spawning areas as groups, within-group comparisons revealed no significant differences in enzyme frequency between samples taken at different times or between fish reared in fresh or salt water ($P > 0.05$). We found a rare homozygous variant allele at locus PGI-2 in one sample but detected no heterozygotes of this allele. From these results we conclude that there were no serious ontogenetic changes in enzyme expression, or changes induced by acclimatization to salt water, and that comparisons among samples of wild fish would not be confounded by these factors.

Ten enzyme loci were polymorphic, 7 above 1% frequency, in the samples of wild fish representing the three life history

TABLE 3. PMI-2 heterozygotes observed and deviation from Hardy–Weinberg for all samples.

	Wild subyearling estuarine smolts	Wild subyearling riverine smolts	Wild yearling riverine smolts	Hatchery subyearling estuarine smolts	Hatchery subyearling riverine smolts	Hatchery saltwater mortalities
Sample size	231	317	35	155[a]	265[a]	26
Heterozygotes						
Observed	95	137	16	51	117	18
Expected	115.4	157.2	17.4	75.6	132.6	12.9
Coefficient of deviation	−0.18	−0.13	−0.08	−0.33	−0.12	+0.40
Hardy–Weinberg equilibrium P (χ^2 test)	<0.01	>0.05	>0.05	<0.01	>0.05	<0.05

[a]Samples combined from two tanks of each group before and after saltwater acclimatization.

types (Table 2). The samples were identically monomorphic at all other loci. Comparison of allele frequencies for the polymorphic loci between year-classes within each life history type revealed only one significant difference ($P < 0.05$) out of 30 comparisons (10 polymorphic enzymes × 3 life history types). We therefore combined samples from different years for each life history type. Juveniles captured at the middle spawning area did not differ significantly at any enzyme locus from subyearling riverine smolts captured during seaward migration. Since the fish captured at the middle spawning area were believed to represent subyearling riverine smolts, we combined these samples with those captured during seaward migration.

The allelic frequencies of AAT-3, MDH-3,4, 6-PGD, and SDH-1 differed significantly among the three life history types. The slow allele of AAT-3 occurred in almost 22% of subyearling estuarine smolts but in less than 10% of yearling riverine smolts. Subyearling riverine smolts were intermediate at 12%. MDH-3,4 occurred in three forms, a fast, an intermediate, and a slow form. The fast and slow forms made up 2.6% of the subyearling estuarine smolt sample, but both forms were absent from the yearling smolts. Subyearling riverine smolts were again intermediate at 0.5%. By contrast, a slow form of 6-PGD occurred only in the yearling riverine smolts. A slow form of SDH-1 occurred in about 14% of subyearling estuarine smolts and yearling riverine smolts but occurred in only 8.7% of subyearling riverine smolts (Table 2).

PMI-2 was highly polymorphic (the rarest allele was 46% of the sample) at similar levels in all samples. This was the only enzyme locus that was not in Hardy–Weinberg equilibrium ($P < 0.05$). Heterozygotes were less abundant than expected in all samples except the sample of fish that died during acclimatization to salt water in the laboratory, and the deficiency was significant ($P < 0.05$) in subyearling estuarine smolts and in hatchery fry that were progeny of spawners from the lower spawning region (Table 3).

Genetic distance was greatest between subyearling estuarine and yearling riverine smolts ($D = 0.0018$) and least between subyearling estuarine and subyearling riverine smolts ($D = 0.0006$). Fixation indices (Fst) were low for all polymorphic loci except 6-PGD (Fst = 0.048; Table 2).

Although the transfer of fry to salt water in the laboratory was not designed to measure salinity tolerance, mortality during the 7-d period of acclimatization to seawater was significantly greater among progeny of spawners from the middle spawning region than among progeny of spawners from the lower spawning region (13.1% compared with 2.5%, $\chi^2 = 20.02$, $P < 0.01$). Mortalities, although low, were significantly greater in both tanks transferred to salt water than in tanks kept fresh ($\chi^2 = 36.5$, $P < 0.05$). After the period of acclimatization to seawater, mortality fell to zero in all tanks.

We analyzed the enzyme systems of 26 juveniles that died during acclimatization to seawater. These were all progeny of spawners from the middle spawning region. Too few fry from the lower region died to make analysis of their enzymes worthwhile. The allelic frequencies at nine polymorphic loci were not significantly different from those in the May 31 sample from the same tank. Although we did not stain LGG in the April 10 sample from this group of hatchery fish, the frequency of the LGG fast allele among mortalities was significantly higher than among wild subyearling riverine smolts, which these hatchery fish are presumed to represent (88.5% compared with 67.6%, $P < 0.05$). At the PMI-2 locus a significant ($\chi^2 = 4.02$, $P < 0.05$) excess of heterozygotes occurred in contrast with all other samples, which were deficient in this heterozygote (Table 3). Although mortality appeared biased toward some alleles, based on this sample, the overall mortality of fry in the laboratory was so low that no changes in enzyme frequency were detected in the samples from surviving fish.

Morphometric differences among the wild samples of the three life history types are consistent with the enzymatic evidence for genetic differences. Five of the measurements (head length, fin length, head depth, body depth, eye diameter) differed significantly among the life history types (Table 4). However, two of these characteristics (fin length, body depth) also differed significantly in their regression coefficients with the covariate. The slope of fin length on fork length was significantly greater among subyearling estuarine smolts than among the other two life history types, while the slope of body depth on fork length was significantly lower among yearling riverine smolts than among the other two life history types (Table 4). The regression coefficient of body weight on fork length also differed among the life history types, the slope for yearling riverine smolts being significantly less than for the other two life history types. The means of all five body measurements increased from subyearling estuarine smolts through subyearling riverine smolts to yearling riverine smolts. The interpretation of adjusted means for those characters in which regression coefficients also differed is problematic. Subyearling estuarine smolts, however, were apparently slimmer with smaller eyes and heads and shorter fins than were the

TABLE 4. Adjusted treatment means, regression coefficients, and F statistics for the analysis of covariance of seven body measurements among wild samples of three life history types of chinook salmon. Fork length was the covariate in all analyses. SYE = subyearling estuarine smolt (N = 44); SYR = subyearling riverine smolt (N = 38); YR = yearling riverine smolt (N = 23). *$P < 0.05$; **$P < 0.01$.

Body measurement	Adjusted treatment mean				Regression coefficient			
	SYE	SYR	YR	F	SYE	SYR	YR	F
Weight (g)	2.31	2.33	2.42	0.46	3.12	3.23	2.39	15.35**
Nose length (mm)	3.58	3.69	3.68	0.78	1.08	0.80	0.91	2.08
Head length (mm)	13.86	14.43	14.71	5.34*	0.93	0.89	0.81	1.71
Fin length (mm)	7.00	8.00	9.88	18.16*	1.00	0.66	0.83	5.02*
Head depth (mm)	6.92	7.74	7.95	23.72*	0.82	0.68	0.68	1.46
Body depth (mm)	10.98	11.07	11.97	4.25*	1.21	1.13	0.71	12.45*
Eye diameter (mm)	3.90	4.26	5.33	48.92*	0.50	0.61	0.36	2.41

riverine fish, and yearling riverine smolts had, relatively, the largest heads and eyes, deepest bodies, and longest pectoral fins.

Discussion

Wild juvenile chinook from the Nanaimo River show enzyme polymorphism comparable with that observed in other chinook populations (Utter and Ebel 1981). The Nanaimo River population is similar to Columbia River and Puget Sound populations in having a very low frequency of PGI-2 variants and no PGM variants. Populations farther south tend to be highly polymorphic at these loci (Kristiansson and McIntyre 1976). The Nanaimo chinook also resembled both spring and fall Columbia River chinook in having a low frequency of variants at the IDH-3, LDH-5, and MDH-3,4 loci (Utter and Ebel 1981; Milner et al. 1981). Allelic frequencies at the PMI-2 locus in the Nanaimo River population were similar to spring run Columbia River chinook. Nanaimo chinook differed from both Columbia River and Oregon chinook populations in having higher frequencies of LGG variants and lower frequencies of SOD variants. It appears that, while Nanaimo chinook are similar in many respects to Puget Sound and Columbia River chinook, they are, nevertheless, distinct from these populations.

Variation in allelic frequencies at four polymorphic loci among wild samples indicated significant genetic differences among all three life history types within the Nanaimo River and that the three types come from distinct subpopulations. Restricted gene flow between subyearling estuarine smolts and the other two types was evident at the AAT-3 and MDH-3,4 loci. Yearling riverine smolts differed from subyearling estuarine and riverine smolts at the 6-PGD locus. Restricted gene flow between subyearling riverine smolts and the other two types was evident at the SDH-1 locus. This degree of differentiation among subpopulations within a small geographic area is greater than would be expected to occur by chance and is comparable with differences in allelic frequency observed over a much wider area in Washington and Oregon (Kristiansson and McIntyre 1976). Genetic distance (Nei 1972) was small between subpopulations, however, as were fixation indices (Hartl 1980) of the polymorphic loci. These results indicate a relatively weak genetic separation of the life history types.

Allendorf and Phelps (1981) suggested that differences in allelic frequency of this magnitude among samples of juveniles from different subpopulations may arise simply due to the finite effective population size of the spawning subpopulations, even if the adults were segregating at random. This kind of sampling error should, however, also have been evident between year-classes of subyearling estuarine and riverine smolts. Yet there were no significant differences between year-classes within a life history type. We conclude, therefore, that the differences in allelic frequencies reflect real genetic differences between the subpopulations producing different juvenile life history types. Allendorf and Phelps (1981) further suggested that the rates of genetic exchange between subpopulations due to migration may be calculated from the allelic frequencies, provided one assumes that the alleles are selectively neutral and their divergence is a consequence of genetic drift. The variations in allelic frequency among the Nanaimo River subpopulations are consistent with a migration rate between subpopulations on the order of 1.5–2% per generation if genetic drift is the principal cause of divergence.

The adaptive significance of electrophoretically detectable genetic variation is still very much a matter of debate. Although there is good correlative evidence for the adaptive significance of some allelic variation (Johnson 1971; Fujino and Kang 1968; Mitton and Koehn 1975) there is equally compelling evidence that much of the electrophoretically detectable variation in fishes is selectively neutral (Aspinwall 1974; Clayton 1981). Assuming a correlation between morphology and enzyme variation, the existence of significant differences in body morphology among the three life history types supports the notion that the electrophoretic differences reflect genetic adaptation to different environments. Among the wild fish, a trend to larger heads, deeper bodies, and longer pectoral fins was correlated with length of freshwater residence. While there is no evidence, as yet, that these traits are heritable in chinook salmon, Riddell et al. (1981) have demonstrated the heritability of similar morphological features in Atlantic salmon. It seems likely that heritability of these traits will be similar in chinook salmon (1–3%).

The deficiency of PMI-2 heterozygotes found in most of the samples (but significant only in the subyearling estuarine smolts and hatchery juveniles from the lower spawning region) should be unstable and lead ultimately to the fixation of the more abundant allele. Two hypotheses may explain the continued existence of such a deficiency. A rare recessive, or null, allele may exist in the population, which produces a nonfunctioning

enzyme in the homozygous state, lethal to the zygote and therefore not detected by electrophoresis of juveniles (Christiansen 1976). In the heterozygous state the gel would not show the band for this allele and the sample would appear homozygous for one of the other alleles, increasing the number of apparent homozygotes yet decreasing the number of real heterozygotes. The size of the observed heterozygote deficiency, however, would require that the allele be present at a frequency of about 0.10, an unlikely frequency for a recessive lethal allele. An alternate, and more credible, hypothesis is that heterozygotes for this locus are selected against during transition to salt water but are at a selective advantage at a later time, possibly during reentry to freshwater to spawn (Christiansen et al. 1977).

To recapitulate, the populaton of chinook in the Nanaimo River is composed of three subpopulations that differ in their age of seaward migration. Rich and Holmes (1928) first postulated that spring and fall run chinook were genetically distinct and produced yearling smolts and subyearling smolts, respectively. The results of this study are consistent with this hypothesis but further suggest that genetic diversity among chinook in the Nanaimo River extends to at least three subpopulations. One subpopulation appears to be adapted to the high-salinity environment of the river estuary from emergence, while two other subpopulations are adapted to the river and lake systems for rearing. These subpopulations are genetically and morphologically distinct and apparently are produced principally from geographically separated spawning areas. The utilization of varied spawning and rearing habitat potentially maximizes the probability of any year-class contributing to future generations and may maximize population fitness by maximizing the overall production of juveniles from any river system.

The existence of this kind of genetically based variability complicates management of a resource, such as chinook, for which there is high demand. Hatcheries currently are the most popular tool for increasing production of chinook salmon to meet recreational and commercial demands for this fish. Helle (1981) discussed the most important general problems arising from the use of hatcheries to propagate species with a naturally high genetic diversity. We would like to emphasize two problems that are particularly relevant in view of the kind of genetic diversity that we have described for chinook. First, constraining juveniles that are genetically subyearling estuarine or yearling riverine type to behave like subyearling riverine type (the norm of hatchery practice in British Columbia) could affect their subsequent survival and thus the success of the hatchery. For example, although subyearling estuarine fish might be of large size when released from a hatchery in June, they may be programmed to remain in the estuary for a long time (their normal behavior pattern), rather than a short time as subyearling riverine smolts normally do. Such a consequence may be occurring in the Campbell River where hatchery chinook remain in the estuary into August, considerably later than wild subyearling riverine smolts in other rivers on the east coast of Vancouver Island (F. Withler, Department of Fisheries and Oceans, Nanaimo, pers. comm.). The Campbell River probably produced a high proportion of subyearling estuarine type fish in its natural state, and it is conceivable that the unusual behavior of the hatchery smolts is due to their normal migratory timing having been disrupted by the hatchery practice. Second, intensive production of only one life history type in hatcheries could lead to destructive overexploitation of unenhanced life history types in an intensive fishery. This, in turn, may cause a general reduction in fitness of surviving wild stocks because the resulting mixture of life history types does not exploit as wide a range of habitats as formerly.

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Homing and Straying in Chinook Salmon (*Oncorhynchus tshawytscha*) from Cowlitz River Hatchery, Washington

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The patterns of homing and straying of spring chinook salmon (*Oncorhynchus tshawytscha*) from the Cowlitz River, Washington, were analyzed, based on coded wire tag recoveries. Out of an estimated escapement of 41 085 chinook salmon, 98.6% returned to Cowlitz River and the rest were recovered in other rivers. Almost all strays were within the Columbia River system, and most were found in the Lewis and Kalama rivers, upstream of Cowlitz River. Straying was positively correlated with age at return and negatively correlated with number of returning salmon.

D'après les récupérations d'étiquettes métalliques codées, on a analysé les régimes de retour aux frayères natales et d'égarement du saumon quinnat (*Oncorhynchus tsawytscha*) de la rivière Cowlitz (Washington). Des remontes évaluées à 41 085 poissons, 98,6 % sont retournés à la rivière Cowlitz tandis que le reste a été capturé dans d'autres rivières. Presque tous les poissons égarés ont été pêchés dans le système du fleuve Columbia et la plupart ont été trouvés dans les rivières Lewis et Kalama, en amont de la rivière Cowlitz. L'égarement était en corrélation positive avec l'âge au retour et en corrélation négative avec le nombre de saumons à la remonte.

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The return of adult Pacific salmon (*Oncorhynchus* spp.) to spawn in the stream they departed as juveniles is common knowledge among fisheries biologists. The most conclusive early study supporting the "parent stream theory" was Foerster's (1936) report that after all the seaward migrating sockeye salmon (*O. nerka*) from Cultus Lake, B.C., had been marked, virtually all adults returning to the lake were marked. Other studies have shown that transplanted juvenile salmon return as adults to the point of release (Donaldson and Allen 1958; Jensen and Duncan 1971). However, there are indications that sizeable numbers of salmon may "stray" or return to non-natal streams (Shapovalov and Taft 1954) and that some salmon may ascend the non-natal river for considerable distances (McLeod and O'Neil 1983).

Interest in salmon homing has generally focussed on the sensory mechanisms underlying migrations on the high seas (Royce et al. 1968; Quinn 1982) and in freshwater (reviewed by Hasler and Scholz 1983). The phenomenon of straying in salmon is generally ignored, and reliable estimates of the actual proportions of salmon that home are virtually nonexistent. The purpose of this paper is to present data on chinook salmon (*O. tshawytscha*) returning to Cowlitz River, Washington, to (1) assess quantitatively the precision of salmon homing, (2) determine the geographic pattern of straying, and (3) investigate life history characteristics of the salmon that home and stray in an effort to understand these phenomena.

Materials and Methods

A tag has been developed for Pacific salmon that consists of a 1-mm-long piece of coded stainless steel wire inserted in the cranial cartilage of young salmon (Jefferts et al. 1963). By convention, the adipose fin is clipped to signal the presence of a coded wire tag (CWT). The use of the CWT system is now common in hatcheries in California, Idaho, Oregon, Washington, British Columbia, and Alaska. Tag codes indicate initiating agency, release site, year, and any treatments the salmon received prior to release. CWT returns from anglers, field crews, and hatchery staff permit coastwide assessment of salmon migrations not possible with any of the early marking techniques.

While it will eventually be possible to analyze CWT returns from many hatcheries, the Cowlitz River Hatchery in Washington (Fig. 1) was selected for analysis now because (1) it has produced substantial numbers of tagged chinook salmon for several years, (2) the hatchery enhances a run of salmon native to the river, eliminating the ambiguities of studying homing in non-native salmon (Bams 1976), (3) there are many hatcheries

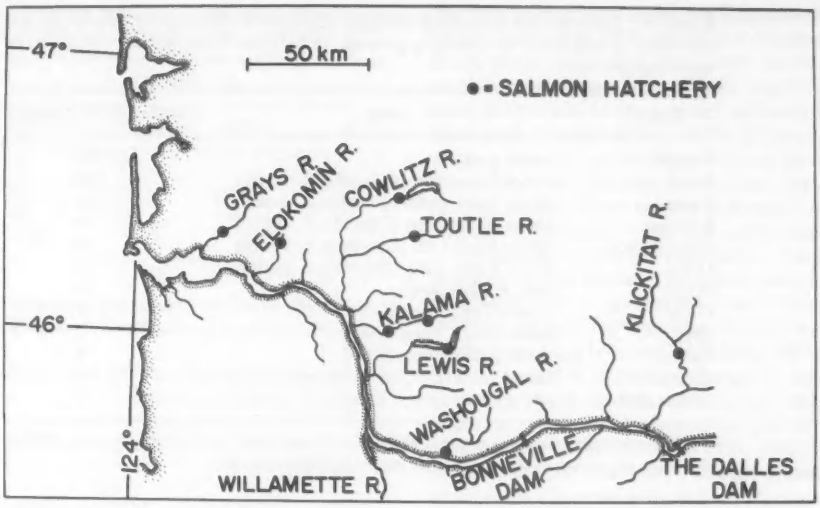

FIG. 1. Lower Columbia River system showing major tributaries and salmon hatcheries.

on nearby tributaries of the Columbia River where tagged salmon are routinely examined, increasing the chances of detecting strays, (4) the heavy sport fishing in this area is sampled by the Department of Fisheries, and (5) the ocean distribution of Cowlitz River chinook salmon is generally known (Wahle et al. 1981).

The data set consists of records on spring chinook salmon spawned in the fall, 1974–77 (referred to as brood years 1974–77), and released after about 470 d of rearing, in March of their 2nd yr. Reliable records were available for returns in 1978–81. Prior to the 1974 brood year and 1978 return year, tagging and recovery efforts were lower and less uniform, so these returns were not examined. Virtually all tag returns were from Washington Department of Fisheries (W.D.F.) hatcheries and W.D.F. surveys of terminal sports fisheries and spawning grounds. This represents the most reliable source of data available to us because all hatcheries were surveyed in all years from 1978 to 1981. In addition, all five British Columbia chinook salmon hatcheries that operated during this period were checked for strays, although records were not available for 1981. Data from the Oregon Department of Fish and Wildlife (O.D.F.W.) included hatchery returns and also returns from sport and test fisheries in certain areas of the Columbia River system. The United States Fish and Wildlife Service (U.S.F.W.S.) provided data on their Columbia River system hatcheries and other hatcheries operated by them in Washington. Additionally, records from some Native Indian facilities were examined by U.S.F.W.S. but on a less consistent basis. Details on the hatcheries of the Columbia River system and the west coast may be found in Wahle and Smith (1979). While it is inevitable that some tagged fish went unsampled, the broad area covered ensures that this was relatively infrequent. Nevertheless, the estimates of straying are necessarily lower than the actual straying rates.

Results

In all, 1.2 million tagged salmon were released, and 24 139 (2%) tags were recovered. Estimates of the total number of tagged fish recovered in commercial and sport fisheries sampled by Washington were derived by multiplying the number of observed recoveries by the inverse of the sampling rate (catch/sample), generally calculated on a monthly basis. Expansion factors for hatchery rack and spawning ground survey returns were derived by dividing the total number of returning fish by the number sampled. W.D.F. estimated the actual total recovery (ocean sport and commercial fishing, river fishing, hatchery returns, and spawning ground surveys) to have been 63 283. Of this total, the estimated return of tagged fish to spawning streams based on stream sport fishing, hatchery returns, and spawning ground surveys was 41 085 (from 18 085 tags read). Only estimated return data were used for analysis.

Of the estimated 41 085 salmon that returned to tributaries, 582 (1.4%) were recovered away from Cowlitz River and the remaining 98.6% were recovered at the Cowlitz Hatchery, caught by sport fishermen just below the hatchery, or found on spawning grounds in the river (Table 1). Most salmon not recovered in Cowlitz River were geographically very close to it (Fig. 1). Of the 582 strays, only 10 (1.7%) were recovered outside the Columbia River system, in tributaries of Puget Sound (Cedar River, Skagit River, and its tributary Cascade River) and the Juan de Fuca Strait (Hoko River). This is consistent with tag returns from ocean commercial and sport fishermen: virtually all marine recoveries indicate movement north along the coast of Washington, east along the Juan de Fuca Strait, and into Puget Sound. Some Cowlitz River chinook salmon are recovered off British Columbia and Alaska, but they seem to rarely migrate south along the coasts of Oregon and California.

Within the Columbia River system, only one salmon was recovered in a tributary below Cowlitz River (Grays River) and one was recovered at Priest Rapids (approximately 550 km upstream of Cowlitz River). The great majority (76.6%) of the strays entered Lewis River and most of the rest (18.6%) entered Kalama River, 30 and 9 km upstream of Cowlitz River, respectively.

Age-class analysis revealed that older salmon strayed more than younger ones (Table 2). However, homing by salmon of different age-classes is confounded by differences in the timing of their return to Cowlitz River. Older salmon, returning after

TABLE 1. Geographical distribution of tagged adult Cowlitz River spring chinook salmon recovered at hatcheries, on spawning grounds, or by sport fishermen near hatcheries or spawning grounds.

River	Area	Calculated total recovery
Cowlitz	Home river	40 503
Lewis	Above home river, Columbia R. system	446
Kalama	Above home river, Columbia R. system	108
Klickitat	Above home river, Columbia R. system	16
Priest Rapids	Above home river, Columbia R. system	1
Grays	Below home river, Columbia R. system	1
Hoko	Juan de Fuca Strait	4
Cedar	Central Puget Sound	4
Skagit	North-central Puget Sound	1
Cascade	North-central Puget Sound	1
Columbia[a]	Above home river, below Bonneville Dam	1
Willamette[a]	Above home river, Columbia R. system	4

[a]These sampling areas were not associated with hatcheries or spawning grounds. These fish were not included when assessing the overall straying rate.

TABLE 2. Relationship between age of Cowlitz River spring chinook salmon and straying.

Age	Calculated total recovery	Strayed	%
Brood year +2	17 495	60	0.34
Brood year +3	3 927	61	1.55
Brood year +4	15 046	299	1.99
Brood year +5	4 560	162	3.55
Brood year +6	57	0	0
Total	41 085	582	1.42

1–3 yr at sea, generally entered Cowlitz River in April and May, as evidenced by sport fishing returns (Fig. 2). However, many salmon released in March returned to the river that summer, primarily in June and July (Fig. 2). These young salmon displayed the greatest fidelity for their home river (Table 2).

Evidence of straying in previous studies has generally been inferred from one-way fish traps that do not permit the salmon to back out and continue their search for home. Thus, it has been unclear whether salmon recorded as strays would have eventually entered their natal river if they had been permitted to do so. In the present study, salmon entering hatcheries other than the Cowlitz cannot be unequivocally classed as strays. However, the 85 salmon that spawned away from the Cowlitz River were strays in the strictest sense.

Analysis of straying to Lewis River (Fig. 3) indicates that Cowlitz chinook salmon enter the river in April and probably remain there during the summer, as shown by sport catches. In the fall, sport catches decline and the salmon spawn or enter Speelyai Hatchery in September.

It is possible to consider differences in homing among brood years (Table 3) and return years (Table 4). While the unequal representation of certain age-classes and brood years confounds analysis, in both cases there is an inverse relationship between the number of salmon recovered and the proportion of strays.

While no Cowlitz River chinook were reported from any O.D.F.W. hatcheries, 353 tagged salmon were recovered from test fisheries or sport fishermen in the Columbia River system. Of these, all but five were recovered below the Cowlitz River and so cannot be considered strays. One salmon was captured in

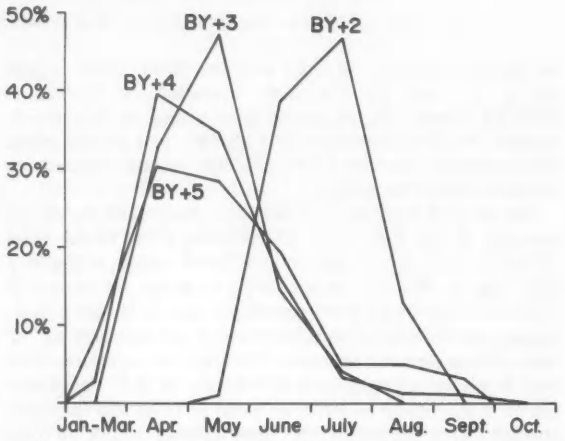

FIG. 2. Timing of spring chinook salmon sport catch in Cowlitz River, separated by age-class. Sample sizes are as follows: brood year +2, $N = 3466$; brood year +3, $N = 2352$; brood year +4, $N = 5461$; brood year +5, $N = 1073$.

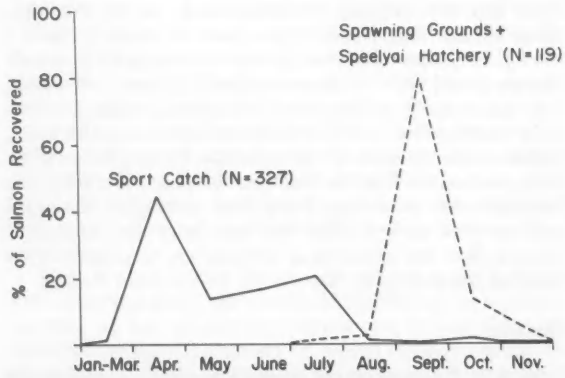

FIG. 3. Timing of Cowlitz River spring chinook salmon recoveries in Lewis River.

TABLE 3. Variation in homing performance of Cowlitz River spring chinook salmon from different brood years (return years 1978–81).

	Brood years			
	1974	1975	1976	1977
Calculated no. recovered	670	4761	33 084	2570
Strays	53	95	327	107
% strays	7.91	2.00	0.98	4.16

TABLE 4. Variation in homing performance of Cowlitz River spring chinook salmon returning in different years (including brood years 1974–77 only).

	Return years			
	1978	1979	1980	1981
Calculated no. recovered	18 069	6134	12 384	4498
Strays	68	147	197	170
% strays	0.38	2.40	1.59	3.78

the main trunk of the Columbia River above the Cowlitz River but below Bonneville Dam. Four salmon were captured in the lower portion of the Willamette River, Oregon, above the Cowlitz River. However, none were reported from any of the hatcheries farther up the Willamette River. While these five fish passed their home river, they were not found near spawning grounds or hatcheries; thus, they cannot be classified as strays in the same sense as fish recovered from W.D.F. facilities or surveys.

Discussion

The first goal of the study was to determine the accuracy of homing in Cowlitz River Hatchery chinook salmon. Of the estimated 41 085 returning salmon, all but 10 were recovered in the Columbia River system, and 98.6% were recovered in the Cowlitz River. While some strays were undoubtedly missed, considering the number of sites where tagged fish could have been detected, this is evidence that the homing response of these fish was very strong. The high level of homing in chinook salmon is consistent with predictions on interspecific variation in homing (Quinn 1984). Other studies of chinook salmon have indicated that this level of homing does not always occur. Snyder (1931) reported 87% homing to Fall Creek, California (though only 39 tagged salmon were recovered). Sholes and Hallock (1979) presented data on chinook salmon released in the Feather River and recovered there and in the American River, California. While the overall homing proportion was 90%, the brood years varied considerably: 99% of brood year 1976 homed vs. only 55% of brood year 1970. Patterns of homing and straying to hatcheries and natural spawning grounds may differ, but there appears to be no comparable large-scale study of homing in wild chinook salmon.

Two rivers, Lewis and Kalama, accounted for 95% of the known strays. While they are close to Cowlitz River, they are not as close as the Toutle River (Fig. 1), which had no reported strays. The eruption of Mt. St. Helens on 18 May 1980 destroyed the hatchery on the Toutle River, so Cowlitz salmon could not have entered there that fall or in 1981, but the reason for their absence in 1978 and 1979 is not clear. Kalama River is 21 km closer to Cowlitz than Lewis, but it received only one fourth as many strays. Lewis is the larger river of the two and tends to be cooler in the summer and early fall (United States Geological Survey 1975, 1979), facts that may influence straying. Caution is required in utilizing the data to explain why strays enter one river and not another. Recorded variation in straying among rivers may be influenced by such ecological factors as temperature, water flow, and turbidity, but it may also be influenced by fishing regulations, access for fishermen, practices of local hatchery personnel, and other factors. While there were more strays in 1981 than in the other return years, 1980 did not have a notably high proportion of strays (Table 4). This is interesting because the 18 May 1980 eruption of Mt. St. Helens resulted in substantial changes in the Cowlitz and lower Columbia rivers during summer and fall 1980 and 1981. Moreover, chinook salmon tended to avoid volcanic ash in an experimental flume (Whitman et al. 1982). The homing data indicate that the addition of foreign material to the Cowlitz River did not prevent salmon from recognizing it and that the repellent nature of the material did not deter them from ascending the home river. However, the Toutle River was more affected by the eruption, and many Toutle River coho (*O. kisutch*) and chinook ascended the Cowlitz River in 1980 and 1981 (W.D.F., unpubl. data, provided by D. Martin, University of Washington).

Straying was about 5–10 times less prevalent among the youngest returning salmon than among the three succeeding age-classes (Table 2). Salmon from the older age-classes were caught at sea, and we may assume that all of them reared outside the Columbia River system. The youngest salmon, however, returned the same year they were released. They were generally released in March, weighing about 100–110 g (20–21 cm fork length). None were reported caught at sea, but at this size they would not be legal to keep, so the absence of reports does not prove that the fish did not enter salt water. When caught by sport fishermen in the Cowlitz River in June and July, they weighed about 400 g (31 cm). Their rapid growth makes it unlikely that they remained in freshwater. The probable scenario is that they left the Cowlitz River in March, briefly entered salt water and fed near the mouth of the Columbia River, and then returned to the Cowlitz River in June, after most of the older fish had arrived. Assuming that they went to the estuary of Columbia River, they faced essentially the same homing problem as the older salmon: which is Cowlitz River? The apparent decline in homing with age suggests that memory loss may play a role, but this tendency is not evident in the data reported by Sholes and Hallock (1979).

In the present study, large numbers of returning salmon were associated with high proportions of homing. In the only comparable study, Sholes and Hallock (1979) monitored the Feather and American rivers for marked salmon over six return years. In the two years with relatively large returns (592 and 689 salmon), 98% of the returning salmon were recovered in their home river. In the four years with smaller returns (48–201 salmon), only 68% homed. It may be that some social factor, either direct contact or chemical stimulation, strengthens the motivation to home. Alternatively, cues aiding home stream selection may themselves be pheromonal, as suggested by Nordeng (1977).

In general, it may be said that homing to the Cowlitz, a large downriver hatchery, was very accurate and that strays were

generally found near the home river. Since most salmon rivers in the Columbia River system are upstream of the Cowlitz River, it is perhaps to be expected that most strays went upstream. Comparison with a rearing facility farther upriver would indicate whether straying salmon tend to pass their natal river rather than stop short of it. It is known that salmon sometimes ascend non-natal rivers, only to return to spawn in their homestream (Ricker and Robertson 1935). Thus, it is particularly important that 85 strays were actually recovered from spawning grounds in other rivers.

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Biochemical Population Genetics of Pacific Halibut (*Hippoglossus stenolepis*) and Comparison with Atlantic Halibut (*H. hippoglossus*)

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The gene products of 35 protein-coding loci were examined for Mendelian variation in three samples of Pacific halibut (*Hippoglossus stenolepis*) and one sample of Atlantic halibut (*H. hippoglossus*). Contingency table analyses of allelic frequencies for five polymorphic loci revealed no significant frequency differences between the Bering Sea and the Gulf of Alaska but detected significant *Ada-2* frequency differences between these regions and Japan. Average genetic distance between the samples of Pacific halibut was 0.0002 ± 0.0007, and gene diversity analyses showed that 98.7% of the total genetic variation was contained within populations, 0.4% was due to differences between the Bering Sea and the Gulf of Alaska, and 0.9% was due to differences between these regions and Japan. These results are consistent with a larval drift, juvenile migration model of population genetic structure where not all juveniles home to their natal areas. Nei's genetic distance between Pacific and Atlantic halibut was 0.162 ± 0.073, and the molecular clock hypothesis suggests that these species became reproductively isolated from one another in the Pliocene between 1.7 and 4.5 million years ago.

On a étudié la variation mendélienne des produits géniques de 35 loci codeurs de protéines, dans trois échantillons de flétan du Pacifique (*Hippoglossus stenolepis*) et un échantillon de flétan de l'Atlantique (*H. hippoglossus*). Des analyses des fréquences alléliques de cinq loci polymorphiques, analyses basées sur un tableau de contingence, n'ont pas révélé de différences significatives de fréquences entre la mer de Béring et le golfe d'Alaska mais elles en ont révélées pour ce qui est des fréquences de *Ada-2* entre ces deux régions et le Japon. La distance génétique moyenne entre les échantillons de flétan du Pacifique s'élevait à 0,0002 ± 0,0007 et des analyses de la diversité génique ont établi les faits suivants : 98,7 % de la variation génétique totale était intra-population, 0,4 % était causée par des différences entre la mer de Béring et le golfe d'Alaska et 0,9 % était imputable aux différences entre ces régions et le Japon. Ces résultats concordent avec le modèle de la structure génétique d'une population où les larves dérivent et les juvéniles migrent mais où les juvéniles ne retournent pas tous aux frayères natales. La distance génétique de Nei entre les flétans du Pacifique et de l'Atlantique s'élevait à 0,162 ± 0,073. Selon l'hypothèse de l'horloge moléculaire, cette valeur porte à croire que les deux espèces sont devenues reproductivement isolées pendant le pliocène, soit il y a 1,7 à 4,5 millions d'années.

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Pacific halibut (*Hippoglossus stenolepis*) is a commercially important flatfish that is distributed from southern California in the eastern North Pacific Ocean, throughout the Bering Sea, to Hokkaido, Japan, in the western North Pacific Ocean (Hart 1973). The migration pattern of halibut, and hence its stock structure, is complex. In the eastern North Pacific Ocean, larval distribution data suggest that eggs and larvae, spawned on the continental shelf in winter, are carried northward by currents off British Columbia and westward by currents off south-central Alaska (Skud 1977). After about 6 mo, these larvae metamorphose into the typical flatfish morphology and settle into a shallow-water demersal existence. Tagging data show that these fish then move into deeper water and begin a southerly migration back toward their natal areas (Skud 1977).

Stock relationships in the Bering Sea appear to be more complicated because in addition to eggs produced by resident Bering Sea adults, eggs and larvae drift into the Bering Sea from the Gulf of Alaska (Skud 1977). Tagging data show that most juveniles remain within the Bering Sea but that some migrate

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into the Gulf of Alaska (Best 1981). These latter fish may have originated in the Gulf of Alaska. Tagging data also show that there is some migration across the Bering Sea; fish tagged off the Kamchatka Peninsula were recovered off Shumigan Island (Best 1981).

A morphologically similar form of halibut, *H. hippoglossus*, inhabits the boreal and subarctic waters of the North Atlantic Ocean from Virginia in the western North Atlantic Ocean to the Bay of Biscay and the Barents Sea on the European side of the Atlantic Ocean (Leim and Scott 1966). There are two views on the taxonomic status of Atlantic and Pacific halibut. Schmidt (1930) considered the Pacific form to be a distinct species, *H. stenolepis*, on the basis of scale morphology, fin ray number, and body shape. However, Vernidub (1936) only gave subspecific or varietal status to Pacific halibut, *H. h. stenolepis*. The present-day geographic distributions of the two taxa do not overlap, and neither form is found in the Arctic Ocean (Leim and Scott 1966; Hart 1973).

In the present study, genetically determined electrophoretically detectable protein variants are used to test for genetic differences between the Gulf of Alaska and the Bering Sea and between these regions and the western North Pacific Ocean. A second goal is to estimate the amount of biochemical genetic distance between Pacific and Atlantic halibut and to determine what the taxonomic status of these taxa should be. Genetic distance is also used to estimate the length of time Pacific and Atlantic halibut have been reproductively isolated from one another.

Materials and Methods

Electrophoresis

Samples of skeletal and heart muscle, liver, and eye, or whole juvenile Pacific halibut were collected near Kayak Island (60°09′N, 46°13′W, July 1981), at several locations in the eastern Bering Sea (transect between 54°00′N, 165°00′W and 55°00′N, 167°00′W, February 1981), and near Hokkaido, Japan (42°34′N, 143°48′E, August 1981). The samples from the eastern Bering Sea were pooled because only a few fish were collected from each location. Tissue samples of Atlantic halibut were collected near Faxaflói, Iceland (64°19′N, 22°32′W, August 1982). The samples were frozen, shipped to Seattle, WA, and held at −20°C until electrophoretic analysis. Samples showing genotypes characteristic of Pacific halibut were stored at −70°C for later comparison with Atlantic halibut.

Extraction procedures and electrophoretic methods followed May et al. (1979), and gel buffer systems are described in Grant et al. (1980). Gels consisted of 13% hydrolyzed potato starch (Electrostarch, Madison, WI, lot 392). Specific enzymes were visualized in the gels after 3–5 h of electrophoresis using staining solutions described by Harris and Hopkinson (1976). The 19 protein systems examined are listed in Table 1. The genetic basis of the banding variants were inferred using four guidelines: (1) comparisons with genetic variation in other teleosts, (2) banding variation should match that predicted from the subunit structure of the protein (Darnall and Klotz 1975), (3) when a gene is expressed in more than one tissue, variant phenotypes should be parallel among tissues, and (4) fit to Castle–Hardy–Weinberg proportions in samples from breeding populations (Fairbairn and Roff 1980). The system of locus and allelic nomenclature followed Allendorf and Utter (1979).

TABLE 1. Enzymatic proteins, locus designations, and tissues used in this study of halibut.

Enzyme	Locus	Tissue[a]
Adenosine deaminase (E.C. 3.5.4.4)	*Ada*	M, H
Adenylate kinase (E.C. 2.7.4.3)	*Ak-1*	M
Alcohol dehydrogenase (E.C. 1.1.1.1)	*Adh*	L
Aspartate aminotransferase (E.C. 2.6.1.1)	*Aat-1*	M
Creatine kinase (E.C. 2.7.3.2)	*Ck-2*	M
	Ck-3	M, H
	Ck-4	E
Esterase (E.C. 3.1.1.1)	*Est-1*	M
	Est-2	M
Glucosephosphate isomerase (E.C. 5.3.1.9)	*Gpi-1*	M
	Gpi-2	M, E
Glyceraldehyde phosphate dehydrogenase (E.C. 1.2.1.12)	*Gap-1*	M
	Gap-2	H
	Gap-3	H, E
Glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.8)	*G3p-2*	M, H
	G3p-3	M
Isocitrate dehydrogenase (E.C. 1.1.1.42)	*Idh-1*	H, M
	Idh-2	L
Lactate dehydrogenase (E.C. 1.1.1.27)	*Ldh-1*	M
	Ldh-2	E
	Ldh-4	E
Malate dehydrogenase (E.C. 1.1.1.37)	*Mdh-1*	M
	Mdh-2	H, E
	Mdh-3	M
Mannosephosphate isomerase (E.C. 5.3.1.8)	*Mpi*	M
Phosphoglucomutase (E.C. 2.7.5.3)	*Pgm-1*	M
	Pgm-2	H, L
Phosphogluconate dehydrogenase (E.C. 1.1.1.44)	*Pgd*	M, L
Phosphoglycerate kinase (E.C. 2.7.2.3)	*Pgk*	M
Peptidase (E.C. 3.4.11)	*La-3*[b]	M
	La-4	M
	Lgg-1[c]	M
	Php-2[d]	M
Superoxide dismutase (E.C. 1.15.1.1)	*Sod*	L
Xanthine oxidase (E.C. 1.2.3.2)	*Xo*	L

[a]M = white muscle, H = heart, L = liver, E = vitreous fluid.
[b]Dipeptide substrate: leucyl-alanine.
[c]Tripeptide substrate: leucyl-glycyl-glycine.
[d]Dipeptide substrate: phenylalanine-proline.

Statistical Procedures

We tested the absolute frequencies of observed phenotypes at each locus for deviations from Castle–Hardy–Weinberg proportions using the log-likelihood-statistic, G, for goodness of fit (Sokal and Rohlf 1969) with degrees of freedom equal to the number of expected phenotypes minus the number of alleles. The significance level of each test was modified to account for the increase in type I error when multiple tests are made on the same samples (Cooper 1968). Comparisons were considered significant if G exceeded the value in a chi-square table associated with a probability of $0.05/n$, where n was the number of loci tested. In this way the overall probability of rejecting H_0 by chance was $1 - (1 - 0.05/n)^n \approx 0.05$.

We used three statistics to analyze the geographic distributions of allelic frequencies. The first was a nested contingency table analysis where G was used to test the null hypothesis of no difference between groups of fish. Only five polymorphic loci

having variant-allele frequencies greater than 0.05 were used to avoid low expected values. The degrees of freedom for each comparison were equal to the number of samples minus one, times the number of allelic classes minus one. Significance levels of these comparisons were modified as above to account for the increase in type I error in multiple tests of the same hypothesis. Thus, rejection criteria associated with a P of $0.05/5 = 0.01$ were used to obtain an overall probability of 0.05.

The remaining two statistics are descriptive and cannot be used to test for significant differences between groups of halibut. Genetic distance, D (Nei 1972), and its standard error (Nei and Roychoudhury 1974), both with corrections for sample size (Nei 1978), were calculated for each pair of samples. All of the allele frequency data, including monomorphic loci, were used for this statistic to facilitate comparison with other teleosts. Gorman and Renzi (1979) and Nei (1978) have shown that unbiased estimates of genetic distance may be obtained from a small number of individuals if the average heterozygosity is low (<0.10) and if a large number of loci are examined. The differences between biased and unbiased estimates are small for sample sizes larger than 50.

Finally, the total gene diversity (heterozygosity of allelic frequencies that have been pooled over samples) was partitioned into its components (Nei 1975) at two levels of population subdivision such that $H_T = H_S + D_{SR} + D_{RT}$, where H_S was the average subpopulation heterozygosity, D_{SR} was the gene diversity due to differences between subpopulations (Bering Sea and Gulf of Alaska), and D_{RT} was the gene diversity due to differences between regions (Bering Sea–Gulf of Alaska and Japan). H_S is a measure of the amount of genetic variation contained within a population and can be thought of as the proportion of individuals in a sample that are heterozygous for an average locus.

Results and Discussion

Genetic Variation

In Pacific halibut, 17 (49%) of the 35 protein-coding loci showed evidence of Mendelian variation, but only five loci, *Aat-2*, *Ada-2*, *La-3*, *Lgg-1*, and *Pgm-2*, had variant-allele frequencies greater than 0.05 and hence were useful for testing hypotheses of population structure. In Atlantic halibut, six (17%) loci were polymorphic with three loci, *Ada-2*, *Gpi-2*, and *Pgm-2*, having variant-allele frequencies greater than 0.05. No significant departures from Castle–Hardy–Weinberg proportions were detected for any of the polymorphic loci. All other proteins examined showed single bands, but not necessarily of the same mobility in the two taxa, and were interpreted to be products of single loci. No differences in gene expression among tissues were found in the two taxa. Descriptions of the polymorphic loci used to describe population structure follow.

Adenosine deaminase

A single polymorphic locus having double-banded heterozygotes and single-banded homozygotes characteristic of monomeric proteins was detected in several tissues but was best visualized in skeletal muscle. These banding patterns correspond to *Ada* variation reported for other flatfishes (Ward and Beardmore 1977; McCafferty 1982; Grant et al. 1983).

Aspartate aminotransferase

Two zones of banding appeared where the products of *Aat-1* migrated cathodally and those of *Aat-2* migrated anodally from the origin. Single-banded homozygotes and triple-banded heterozygotes were observed for *Aat-2* and were best visualized in skeletal muscle. Similar polymorphisms characteristic of a dimeric protein have been reported for other flatfishes (Ward and Beardmore 1977; McCafferty 1982).

Glucosephosphate isomerase

Three zones of banding were observed for *Gpi*. The least anodic zone had single- and triple-banded phenotypes and was interpreted to represent a single polymorphic locus. The most anodic zone also had single- and triple-banded phenotypes, presumably coded by a single locus. Single, double, or triple bands of intermediate mobility were associated with specific phenotypes in the other two zones and were interpreted to be interlocus heterodimeric bands. Gene expression for two loci having similar phenotypes has been observed in Atlantic halibut (Mork and Haug 1983) and in other flatfishes (Ward and Beardmore 1977; Fairbairn 1981a, 1981b; Grant et al. 1983).

Peptidases

The products of several peptidase loci appeared using different di- and tri-peptide substrates. Three zones of activity were observed using leucyl-tyrosine where the most anodic zone, *La-3*, had single-banded homozygotes and double-banded heterozygotes characteristic of a monomeric protein. A similar locus has been reported for Pacific herring, *Clupea pallasi* (Grant 1981), but not for other flatfishes. Using leucyl-glycyl-glycine, a single zone of activity, *Lgg*, was observed having single- and triple-banded phenotypes characteristic of a dimeric protein. Similar dimeric phenotypes have been reported for other flatfishes (Grant et al. 1983). The last set of bands was detected using phenylalanine-proline. The least anodic zone of activity was too weak to score routinely, but the more anodic zone, *Php-2*, could be visualized in several tissues. All of the peptidases were best visualized in skeletal muscle tissue.

Phosphoglucomutase

Two zones of banding appeared to represent the products of two loci. *Pgm-1* migrated cathodally from the origin and was most strongly expressed in skeletal muscle. The products of *Pgm-2* were anodic and appeared most intense in heart and liver tissues. *Pgm-2* was polymorphic with six alleles. Each primary band was accompanied by a less intense anodic band. Similar satellite bands have been reported for *Pgm* in other flatfishes (Purdom et al. 1976; Fairbairn 1981a). Using isoelectric focusing, Mork and Haug (1983) reported only a single *Pgm* locus in Atlantic halibut that had a pI of 8.7. This locus probably corresponds to *Pgm-1* in the present study. The absence of *Pgm-2* in their study is most likely due to differences in electrophoretic methods between the studies rather than to differences in gene expression between the taxa.

Population Structure of Pacific Halibut

Allelic frequencies of 21 polymorphic loci for three samples of Pacific halibut and one sample of Atlantic halibut are presented in Table 2. Contingency table analyses of allelic frequencies for *Aat*, *Ada*, *La-3*, *Lgg*, and *Pgm-2* did not reveal significant differences between the Gulf of Alaska and the Bering Sea samples. However, one significant difference for *Ada* ($G = 12.4$, df = 2, $P < 0.01$) was detected between the Japanese sample and the pooled frequencies of the Gulf of

TABLE 2. Allelic frequencies of protein variants of halibut. Alleles are designated by their mobilities relative to the most common allele of Pacific halibut.

Locus	Allele	Pacific: Bering Sea[a]	Pacific: Gulf of Alaska[b]	Pacific: Japan[c]	Atlantic, Iceland[d]
Aat-1	90	0.392	0.309	0.315	0.010
	100	0.608	0.684	0.671	0.990
	107	—	0.007	0.014	—
Ada	74	—	—	—	0.948
	88	0.217	0.250	0.171	0.010
	100	0.617	0.618	0.800	0.042
	105	—	0.007	—	—
	116	0.150	0.110	0.029	—
	120	0.016	0.015	—	—
Adh	−100	1.0	1.0	1.0	—
	90	—	—	—	1.0
Est-1	94	—	0.016	0.014	—
	100	1.0	0.984	0.986	1.0
Gpi-1	−50	—	0.007	—	1.0
	−100	1.0	0.993	1.0	—
Gpi-2	95	—	0.014	—	0.041
	100	1.0	0.986	1.0	0.949
	107	—	—	—	0.010
G3p-3	70	0.008	—	—	—
	100	0.992	1.0	1.0	1.0
Idh-1	100	0.992	1.0	0.957	1.0
	140	0.008	—	0.043	—
Idh-2	84	0.017	0.015	—	—
	88	—	—	—	0.010
	100	0.958	0.956	1.0	0.990
	117	0.025	0.029	—	—
La-1	100	1.0	1.0	1.0	—
	95	—	—	—	1.0
La-3	60	0.059	0.037	0.014	—
	85	0.017	0.022	—	—
	100	0.873	0.934	0.972	1.0
	107	0.051	0.007	0.014	—
Lgg-1	93	0.017	0.022	—	—
	100	0.908	0.971	1.0	1.0
	107	0.067	0.007	—	—
	112	0.008	—	—	—
Mdh-1	100	1.0	1.0	0.986	1.0
	−150	—	—	0.014	—
Mdh-2	20	—	—	—	0.010
	100	1.0	1.0	1.0	0.980
	130	—	—	—	0.010
Mpi	97	0.008	—	—	—
	100	0.984	1.0	1.0	1.0
	108	0.008	—	—	—
Pgd	88	0.008	0.007	—	—
	100	0.984	0.993	1.0	1.0
	105	0.008	—	—	—
Pgm-1	−80	—	—	—	0.011
	−90	—	—	—	0.989
	−100	1.0	1.0	1.0	—
Pgm-2	63	0.008	0.007	0.029	—
	80	0.017	0.037	0.029	—
	92	0.350	0.316	0.271	0.378
	95	0.025	0.044	—	—
	100	0.550	0.418	0.671	0.643
	113	0.050	—	—	—
Php-2	85	—	—	—	0.032
	95	0.008	—	—	—
	100	0.984	0.986	0.986	0.986
	106	0.008	0.014	0.014	—
Sod	97	0.016	—	—	—
	100	0.984	1.0	1.0	1.0
Xo	97	0.008	—	—	—
	100	0.984	0.993	1.0	1.0
	105	0.008	0.007	—	—

[a]N = 60, except *La-3* (59).
[b]N = 68, except *La-3* (67).
[c]N = 35.
[d]N = 49, except *Aat* (48), *Ada* (48), *Idh-1* (47), *Pgm-1* (47), and *Php-2* (47).

Alaska and the Bering Sea samples. The genetic distance between all pairs of samples averaged 0.0002 ± 0.0007, and none of the D's were significantly different from zero. The gene diversity analysis showed that 98.7% of the total gene diversity was contained, on average, within populations, 0.4% was due to differences between the Gulf of Alaska and the Bering Sea, and 0.9% was due to differences between the latter two samples and the Japanese sample.

These results show that juvenile Pacific halibut of the Bering Sea and the Gulf of Alaska are not genetically distinct from one another. Even though adults were not sampled, it is unlikely that a genetically distinct stock of adults resides in the Bering Sea, especially in view of the low amount of transoceanic divergence. The results further suggest that Pacific halibut are not subdivided into oceanic races as are other North Pacific fishes, e.g. Pacific herring (Grant and Utter 1984) and yellowfin sole, *Limanda aspera* (Grant et al. 1983). Unlike halibut, these fishes show genetic racial differences where the between-race genetic distances are an order of magnitude greater than the among-population genetic distances within each race. Such differences if they existed in Pacific halibut would still be apparent even with the relatively small sample sizes used in the present study.

The results of Tsuyuki et al. (1969) also confirm the hypothesis that there is little genetic divergence among Pacific halibut populations of the Gulf of Alaska and the Bering Sea. They examined genetic variation at a single transferrin locus in juvenile halibut collected at 10 locations extending from Vancouver Island to the northeastern Bering Sea. Although they interpreted their allele frequency data to show that there was a distinct population of halibut in southeastern Alaska, a reexamination of their results suggests that the Gulf of Alaska populations are genetically homogeneous. They reported, correctly, that a contingency table analysis of alleles by samples was not significant ($\chi^2_{26} = 33.6$, $P = 0.18$). They were therefore not justified in making a posteriori tests based on the data themselves. They were further incorrect in using the chi-square values for each row (locations) as a statistic with degrees of freedom equal to two to test the hypothesis that the frequencies at each location were not different from the overall average. A gene diversity analysis of their data shows that 99.6% of the transferrin variation was contained, on average, within the samples, 0.4% was due to differences among populations, and 0.09% was due to differences between the Bering Sea and the eastern North Pacific Ocean.

One weakness of the present study and of Tsuyuki et al. (1969) is that only juveniles were sampled and, therefore, it was not possible to test hypotheses of adult population genetic structure. Nonetheless, the results of both studies are consistent with a larval drift, juvenile migration model where not all juveniles home to their natal areas. The genetic data, together with tagging and larval distribution data, show that the populations of Pacific halibut in the eastern North Pacific Ocean and Bering Sea are interrelated and underline the importance of managing the Pacific halibut fishery in these areas as one unit.

A recent study by Mork and Haug (1983) suggests that Atlantic halibut populations also show little divergence. They examined genetic variation for two polymorphic proteins in three small samples from the coast of Norway and reported significantly different allelic frequencies for an unidentified protein. However, if the probability modification to account for the increase in type I error in multiple comparisons of the same sample is applied to these results the heterogeneity is not significant. The frequency data for the *Gpi-2(100)* allele in the sample of Atlantic halibut in the present study can be compared with their allele frequency data for an indication of stock structure on a larger geographic scale. They found an average frequency of 0.91 for the Norwegian coast ($N = 79$) as compared with 0.95 ($N = 47$) for the Icelandic sample.

Divergence between Pacific and Atlantic Halibut

Pleuronectiform flatfishes are thought to have evolved in the Pacific Ocean in the early Tertiary Period because of their greater present-day diversity in the Pacific Ocean (Nikol'skii 1954). Migration into the Atlantic Ocean of cold-adapted fishes such as halibut was not possible until the Bering Seaway opened in the late Miocene or Pliocene. The date of the seaway's formation has been inferred from the biostratigraphy of molluskan deposits in the North Pacific and Atlantic oceans. The earliest suggestion of strata having common species is in the late Miocene about 10–12 million years ago (MacNeil 1965; Hopkins 1972). However, Gladenkov (1979) and Herman and Hopkins (1980) believed that a massive invasion of Pacific mollusks into the North Atlantic Ocean 3.0–3.5 million years ago marked the initial opening of the Bering Strait.

According to the molecular clock hypothesis, the number of amino acid differences between related taxa is proportional to the amount of time since they shared a common gene pool. Nei (1972) theorized that a D of 1.0 represented a divergence time of about 5 million years. However, empirical data suggest that this calibration underestimates divergence times. Correlation of D with albumin immunological distance (AID) has been used most to calibrate D with time. Wyles and Gorman (1980) summarized calibrations for 474 related pairs and found that, on average, a D of 1.0 corresponds to 35.6 AID units. Carlson et al. (1978) correlated estimates of divergence time from paleontological data with AID for 25 species pairs of mammals and found that 1 AID unit represented 1.85 million years of separation ($r = 0.97$). Even though this calibration did not include lower vertebrates, it appears to fit well with AID and divergence times between frogs where plate tectonics were used to estimate the times of separation of frogs on different continents (Maxson et al. 1975). Thus, a D of 1.0 represents about 19 million years of continuous separation. However, Grant (1984) has cautioned that an estimate of a single point in time has little accuracy because of errors associated with estimating paleontological events, errors in the measurements of AID and D, and because of possible errors in the assumptions of the molecular clock itself (Korey 1981). At best, D with its standard error can only be used to estimate a period of time when divergence most likely began.

The average D between samples of Pacific and Atlantic halibut was 0.162 ± 0.073 and, using the above calibrations, suggests that ancestors of Atlantic halibut invaded the North Atlantic Ocean between 4.5 and 1.7 million years ago. This period includes the Pliocene but not the Miocene date of the formation of the Bering Strait.

Genetic distance can also be used to estimate the amount of taxonomic divergence between taxa. Shaklee et al. (1982) reviewed the electrophoretic literature for a large number of marine and freshwater fishes and found that D between conspecific populations averaged 0.05 (range 0.002–0.065), between related species averaged 0.30 (0.025–0.609), and between related genera averaged 0.90 (0.580–1.21). Thus, the estimate of biochemical distance between Pacific and Atlantic halibut is within the range of genetic distances between species and confirms the present practice of treating these taxa as species.

Genetic data are also available for Pacific and Atlantic populations of Greenland halibut (*Reinhardtius hippoglossoides*). Fairbairn (1981a) estimated D between samples from the Bering Sea and the western North Atlantic Ocean to be 0.098 and recommended that these two forms be elevated to subspecific standing. However, only data from two polymorphic loci were used to estimate D, but since data for monomorphic loci must also be included (Nei 1972), this distance cannot be used to estimate dates of divergence or taxonomic status. The estimate of D including all of the data for 16 loci presented in that paper is 0.013 ± 0.010 and is typical of divergence between conspecific populations. This low amount of genetic divergence suggests that, unlike Pacific and Atlantic halibut, there has been recent gene flow across the Arctic Ocean.

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Zooplankton Community Structure, but not Biomass, Influences the Phosphorus–Chlorophyll *a* Relationship[1]

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Simultaneous observations were made of total phosphorus (TP), chlorophyll *a* (Chl *a*), and zooplankton biomass and community structure at 12 sites in the Eastern Townships (ET) region of Quebec to determine if zooplankton parameters reduced residual variation in the TP–Chl *a* relationship. Similar data from three stations in Lake Memphremagog and the literature were also analyzed. Zooplankton biomass was not significant in explaining residual variation in the TP–Chl *a* relationship of any data set studied. The mean body size of cladocerans was also not a significant additional variable in a TP–Chl *a* model based on the seasonal mean values in the ET lakes, but cladoceran body size was a significant factor in 13 lakes of the Indian River district of New York. The difference between these results is attributed to the rarity of large daphnids in the ET lakes. An index of zooplankton community structure derived from the slope of log abundance–log weight regressions was significant in explaining residual variation in TP–Chl *a* relationships for the among- and within-lake data and improved the predictive capability of TP–Chl *a* models. Lakes with higher concentrations of macrozooplankton relative to microzooplankton have less Chl *a* per unit TP. These results extend the generality of the hypothesis that large zooplankton differentially reduce Chl *a* relative to TP. In the ET lakes the effect of zooplankton size structure, however, was weak and this suggests that manipulation of zooplankton community structure to manage algal biomass may be of limited value in many lakes.

On a réalisé des observations simultanées du phosphore total (PT), de la chlorophylle *a* (Chl *a*) ainsi que de la biomasse et de la structure de la communauté zooplanctonique à 12 endroits de la région des Cantons de l'Est (CE) (Québec), dans le but de déterminer si les paramètres relatifs au zooplancton réduisent la variation résiduelle dans la relation entre le PT et la Chl *a*. On a aussi analysé des données semblables, recueillies à trois stations du lac Memphrémagog et dans les ouvrages publiés. La biomasse du zooplancton ne contribuait pas beaucoup à expliquer la variation résiduelle dans la relation PT–Chl *a* de toutes les séries de données étudiées. De plus, la taille moyenne du corps des cladocères n'était pas une variable supplémentaire importante dans le modèle Pt–Chl *a* basé sur les valeurs saisonnières moyennes dans les lacs des CE, mais la taille du corps des cladocères constituait un facteur significatif dans 13 lacs du district de la rivière Indian (New York). La différence entre ces résultats est imputable à la rareté de grosses daphnies dans les lacs des CE. Un indice de la structure de la communauté zooplanctonique, tiré de la pente des régressions abondance (log)–poids (log) expliquant en grande partie la variation résiduelle des relations PT–Chl *a* pour les données intra-lacustres et inter-lacustres et améliorait la capacité prédictive des modèles PT–Chl *a*. Les lacs possédant des hautes concentrations de macrozooplancton par rapport au microzooplancton ont moins de Chl *a* par unité de PT. Ces résultats étendent la généralité de l'hypothèse selon laquelle le gros zooplancton réduit d'une façon distinctive la Chl *a* par rapport au PT. Dans les lacs des CE, l'incidence de la structure de taille du zooplancton était toutefois faible et ce fait porte à croire que la manipulation de la structure de la communauté zooplanctonique dans le but de gérer la biomasse algale peut avoir une valeur limitée dans de nombreux lacs.

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Empirical models combining aspects of nutrient loading, nutrient standing stocks, and lake morphometry have been used successfully to predict algal biomass among lakes (Sakamoto 1966; Dillon and Rigler 1974; Vollenweider 1976; Jones and Bachmann 1976; Oglesby and Schaffner 1978; Canfield and Bachmann 1981; Smith 1982). These models have also been successful in predicting annual changes in algal biomass within lakes during restoration in some but not all cases (Smith and Shapiro 1981). All of the current models rely ultimately upon regressions of the dependent variable, chlorophyll *a* (Chl *a*), on the independent variable, total phosphorus (TP); however, the confidence limits associated with the predictions of these regressions are wide (Dillon and Rigler 1974), and there has been a recent emphasis on analyzing other variables that may reduce this uncertainty (Smith 1982; Hoyer and Jones 1983).

Shapiro (1980a, 1980b) has argued that biological factors have an impact on the ratio of Chl *a* to TP. The community composition and biomass of zooplankton have been proposed as factors that might be managed to ameliorate the effects of

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cultural eutrophication without expensive programs to reduce nutrient loading. Evidence from (1) repeated observations of single lakes over a number of years (Edmondson and Litt 1982), (2) observations of lakes after fish kills (Schindler and Comita 1972; Andersson et al. 1978), and (3) whole lake experiments (Hrbáček et al. 1961; Stenson et al. 1978; Shapiro and Wright 1984) indicates that when large cladoceran zooplankton become abundant in lakes the quantity of Chl is reduced independently of changes in TP.

If these results are general for a wide range of lakes, then the biomass and community composition of zooplankton should emerge as significant additional independent variables in TP–Chl models. High biomasses of zooplankton should be associated with underestimates of Chl *a* from the predictions of TP–Chl *a* models. Similarly, based on evidence reviewed by Shapiro (1980a, 1980b) and Shapiro and Wright (1984), lakes dominated by large zooplankton should have less Chl per unit TP than lakes dominated by small zooplankton.

The purpose of this study was to test these two hypotheses within and among lakes. Total zooplankton biomass and community size structure as well as TP and Chl *a* were measured during the ice-free season in a set of Quebec lakes and within Lake Memphremagog. A second set of tests was performed using data collected from the literature. The rationale for employing within- and among-lake data was to test for differences in the scale at which zooplankton might be important in determining the ratio of Chl *a* to TP. In addition, hypotheses for the among-lake data were tested both for all the data and the seasonal mean values only. This was done to determine if zooplankton influence is apparent for general among-lake differences (mean values) or at the scale of seasonally varying events common among lakes (all the data).

Methods

Study Area

The study sites were located in the Eastern Townships (ET) region of Quebec approximately 100 km east of Montreal. Sites were chosen to represent a range of morphometric and trophic conditions (Table 1). Each site was sampled monthly during the ice-free season (May through September) of 1982. A single station was sampled in each lake except Lake Memphremagog where three stations were sampled along a nutrient gradient that occurs on the long axis of the lake (Peters 1979). The Lake Memphremagog stations were treated as separate water bodies representing each major basin in the lake (see Ross and Kalff 1975; Peters 1979). Because differences in TP and Chl *a* concentrations were relatively small between basins (Table 1), the Lake Memphremagog stations were also used to form a separate within-lake data set including samples from 1981 and 1982. These data were considered appropriate as a test for the effects of zooplankton due to seasonal variation within a system.

Sampling

An integrated whole-water sample was taken with a plastic tube (2-cm diameter) to a depth within 1 m of the bottom at each site. A portion of the tube sample was preserved with $HgCl_2$ and stained with bromophenol blue to sample planktonic ciliates (Pace and Orcutt 1981). Triplicate 50-mL subsamples of the tube sample were oxidized with potassium persulfate under pressure (Menzel and Corwin 1965) and then analyzed for TP using the ascorbic acid modification of the molybdenum blue technique (Strickland and Parsons 1972). A second set of triplicate subsamples (50–200 mL) from the tube sample were filtered on glass fiber filters under low vacuum pressure and frozen. Chl *a* corrected for phaeopigments was subsequently measured for these filters by the acetone extraction technique using a fluorometer (Strickland and Parsons 1972).

Triplicate integrated zooplankton samples were taken with an open diaphragm bilge pump connected to a PVC hose (2.54-cm diameter). Water was drawn through the pump while the hose was retrieved through the water column, and the outflow was passed through either a 75-μm mesh net to sample macrozooplankton (the rotifer *Asplanchna*, postnauplier copepods, and cladocerans) or through a 35-μm sieve to sample the microzooplankton (testate and colonial ciliates, rotifers, and copepod nauplii). Vertical net hauls (0.5-m net diameter, 75-μm mesh) were used to sample the macrozooplankton for the May sampling series in 1982 and for the Lake Memphremagog data in 1981. The net was "calibrated" at each station either by pooling a vertical series (1-m intervals) of Schindler trap samples or with the pump. The mean net haul values were then corrected by assuming that the trap or pump sampled with a 100% efficiency.

Zooplankton were anesthetized with carbonated water. Macrozooplankton were then preserved in a sucrose–formalin solution (Haney and Hall 1975), while microzooplankton were fixed in $HgCl_2$ (Pace and Orcutt 1981).

Zooplankton Counting and Biomass Estimation

To determine densities, at least 400 individuals were counted in subsamples of each replicate microzooplankton and macrozooplankton sample. Microzooplankton were counted with an inverted microscope at 120× magnification. Macrozooplankton samples were counted with a dissecting microscope (37.5×).

To estimate weight, length measurements were made of the most abundant taxa for each sampling date. Mean lengths were then converted to mean dry weights using published length–dry weight regressions (McCauley 1984) and volumetric formulae in the case of rotifers (Ruttner-Kolisko 1977) and larger ciliates (Pace 1982). For many taxa of rotifers and ciliates, the volumetric formulae also required measuring the widths of organisms. All of the microzooplankton measurements were made with the inverted microscope to the nearest 2.3 μm for ciliates and 4.6 μm for rotifers and nauplii. Macrozooplankton were measured using the system described by Sprules et al. (1981), which consists of a dissecting microscope with a viewing screen and an electronic caliper linked via an analog-digital converter to a microcomputer. Additional macrozooplankton measurements were made with the dissecting microscope (37.5×) to the nearest 0.026 mm, except for the small cladocerans *Bosmina* and *Chydorus*, which were measured at 75× magnification to the nearest 0.013 mm. Generally, 20–25 animals were measured for the two or three most abundant taxa in each size fraction, and 10 animals were measured in the case of rarer taxa. The biomass of each taxon for which a size measurement was made was calculated by multiplying the mean density by the estimated weight. The biomass of rotifers, nauplii, postnauplier copepods, and cladocerans was then determined by summing across taxa within each taxonomic category.

The biomass and density of ciliates was determined by settling 5–50 mL of the whole-water sample and counting and

measuring each cell encountered using the Utermöhl inverted microscope method (Lund et al. 1958). At least 50 individuals were counted and sized (maximum length and width to the nearest 2.4 μm) in each sample. Cell volumes of the ciliates were estimated by assuming that all cells approximated prolate spheriods and by converting the calculated volumes to dry weight using the empirically determined conversion factor for ciliates of 0.2793 $pg \cdot \mu m^{-3}$ (Gates et al. 1982).

Indices of Community Structure

The structure of the total zooplankton community may be characterized by regressing the $\log_{10}$ of taxon abundance against the $\log_{10}$ of taxon mean weight. Holtby et al. (L. Holtby, Pacific Biology Station, Nanaimo, B.C., pers. comm.) have applied this approach by treating the zooplankton as particles of known mass according to their size and then measuring the distribution of particle sizes with automatic counting equipment. This yields a regression with a negative slope that provides a quantitative measure of the relative importance of large and small zooplankton (Holtby et al. (L. Holtby, pers. comm.)). It was not possible to treat the zooplankton as individual particles in this study because of the method of data analysis I used. Instead, the abundances of the taxa that were used to calculate biomass were regressed against the estimated mean weights (Fig. 1). Extremely negative slopes represented communities dominated by small zooplankton (Fig. 1B), while communities dominated by larger zooplankton had more positive slopes (Fig. 1A).

Since hypotheses concerning the effect of zooplankton on TP and Chl *a* have emphasized the importance of large crustacean zooplankton (particularly cladocerans), a second measure of community structure was derived by determining the mean size of cladocerans and copepods. One hundred crustaceans and a minimum of 50 cladocerans were measured randomly as encountered for each sampling date using the microscopic methods described above. Daphnids were measured from the top of the eye to the base of the tail spine to avoid confounding effects of differential helmet development. Total carapace length was measured for all other cladocerans, and copepods were measured from the anterior margin of the head to the end of the caudal rami (excluding setae).

Literature Data

The data set of Hanson and Peters (1984) was used to further test the effect of zooplankton biomass on the TP–Chl *a* relationship. This data set consists of observations from 49 nonacidic lakes (pH > 5.5) and includes "growing season" means of epilimnetic TP, epilimnetic Chl *a* (micrograms per litre), and crustacean biomass (micrograms dry weight (dw) per litre). The community structure effect was also examined with data derived from Mills and Schiavone (1982). These workers report TP, Chl *a*, and mean body lengths for cladocerans and "all zooplankton" (cladocerans and copepods) for 13 lakes in the Indian River district of New York. The data are averages of collections made in August 1979 and 1980.

Statistical Procedures

The effect of zooplankton biomass and community structure was tested using stepwise multiple regression (Draper and Smith 1981). TP was entered as the first independent variable to predict the dependent variable Chl *a*. Zooplankton were considered to have a significant effect on the relationship if as a second independent variable the partial *F*-value associated with zooplankton was significant at the 0.05 level. Partial sums of squares values for these *F*-tests reflected the increase in the residual (error) sums of squares if the independent variable in question were removed from the model (SAS Institute Inc. 1982). Except in the case of community structure indices, analyses were performed using log transformed values to stabilize the variance. Because the data were log normal, geometric mean values were used as the best estimates of the ice-free season means for both nutrient and biotic variables. Computations were carried out using the Statistical Analysis System (SAS) programs (SAS Institute Inc. 1982).

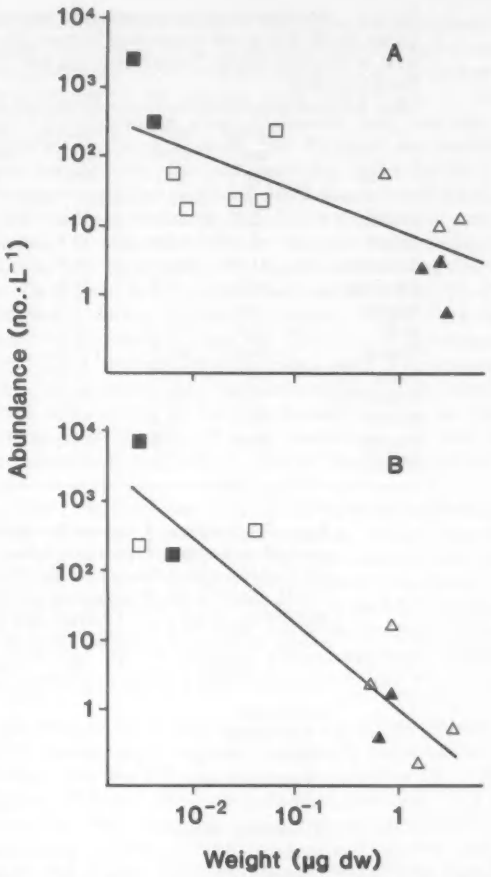

FIG. 1. Log abundance–log weight regressions for (A) Brome Lake in May (model: log abundance = 0.91 − 0.59 log weight, $F = 14.0$, $P = 0.0033$, $r^2 = 0.56$) and (B) Memphremagog–Central in August (model: log abundance = 0.01 − 1.24 log weight, $F = 33.9$, $P = 0.0003$, $r^2 = 0.79$). ■, ciliates; □, rotifers; ▲, cladocerans; △, copepods.

Results

Seasonal average TP concentrations in the ET sites ranged from 3.4 to 55.7 $\mu g \cdot L^{-1}$ in 1982 (Table 1). These sites, therefore, represented a range of trophic conditions including at either extreme oligotrophic (e.g. Bowker, Orford) and

TABLE 1. Morphometric characteristics and mean values of limnological variables for the ET study sites in 1982. Values for TP, Chl *a*, and zooplankton biomass (dw = dry weight) are geometric means. Cladoceran length is the mean of the mean lengths for each sampling date. North, Central, and South represent the three stations in Lake Memphremagog.

Site	Surface area (km^2)	Mean depth (m)	Maximum depth (m)	Secchi depth (m)	TP ($\mu g \cdot L^{-1}$)	Chl *a* ($\mu g \cdot L^{-1}$)	Zooplankton biomass ($\mu g\ dw \cdot L^{-1}$)	Cladoceran length (mm)
Bowker	2.3	24.0	59.0	6.5	3.37	1.15	66.4	0.649
Orford	1.3	17.9	37.5	5.2	5.20	1.43	124.4	0.807
Brompton	11.9	12.5	42.3	2.7	6.19	1.90	90.5	0.763
Lovering	4.6	9.7	24.9	3.2	6.97	2.47	116.6	0.751
Argent	—	—	17.0	2.5	10.50	2.40	147.7	0.696
North	20.4	14.7	33.5	3.5	8.59	3.59	203.9	0.797
Central	24.6	44.3	117.0	3.4	9.72	4.45	200.3	0.548
South	56.1	6.2	12.8	2.9	12.56	5.40	221.5	0.645
Massawippi	17.9	40.2	85.7	2.7	12.46	3.82	254.1	0.368
Brome	14.5	5.8	12.8	2.7	14.43	3.47	217.1	0.816
Magog	10.8	9.8	19.2	1.9	46.98	15.53	489.3	0.606
Waterloo	1.5	2.9	4.9	1.0	55.70	28.61	373.0	0.860

TABLE 2. Analyses of variance for stepwise regression models to predict Chl *a* from TP and zooplankton biomass (ZB) for (A) all data for the ice-free season of 1982 in the ET sites, (B) the 1982 ice-free seasonal means for the ET sites, (C) the 49-lake data set of Hanson and Peters (1984), and (D) Lake Memphremagog only, 1981 and 1982. Coefficients of determination are from the univariate (r_1^2), TP alone, and multivariate (r_2^2) models for each case.

Source	df	SS	*F*	*P*	r_1^2	r_2^2
(A) All data						
Full model	2	8.073	86.49	<0.0001	0.761	0.762
Partial (TP)	1	3.740	80.14	<0.0001		
Partial (ZB)	1	0.013	0.29	0.5943		
Residual	54	2.520				
(B) Seasonal means						
Full model	2	1.660	61.95	<0.0001	0.932	0.932
Partial (TP)	1	0.215	16.07	0.0031		
Partial (ZB)	1	0.001	0.06	0.8128		
Residual	9	0.121				
(C) Literature data						
Full model	2	13.479	53.40	<0.0001	0.691	0.704
Partial (TP)	1	2.499	19.80	<0.0001		
Partial (ZB)	1	0.248	1.96	0.1683		
Residual	45	5.679				
(D) Lake Memphremagog						
Full model	2	0.170	7.45	0.0036	0.414	0.415
Partial (TP)	1	0.163	14.32	0.0011		
Partial (ZB)	1	0.000	0.04	0.8403		
Residual	21	0.239				

eutrophic (e.g. Magog, Waterloo) lakes. Morphometries also varied for the study lakes with surface areas ranging from 1 to 56 km^2 and mean depths from 3 to 44 m (Table 1). Morphometric parameters were not related to trophic conditions. For example, TP was not correlated with either lake area ($r = -0.175$, $P = 0.608$) or mean depth ($r = -0.392$, $P = 0.233$). TP was a good univariate predictor of algal biomass as measured by Chl *a* (Fig. 2). Regressions were highly significant both for all the data collected in 1982 ($F = 175.0$, $P < 0.0001$, $r^2 = 0.76$, $n = 57$) and for the mean values for the ice-free season from each lake ($F = 136.7$, $P < 0.0001$, $r^2 = 0.93$, $n = 12$).

Total zooplankton biomass did not explain a significant amount of the residual variation in the TP–Chl *a* relationship either for all the ET data or the seasonal mean values (Table 2). When zooplankton biomass was partitioned into either taxonomic (ciliates, rotifers, crustaceans) or size-class (micro- and macro-zooplankton) groups, a similar result was obtained. Neither total zooplankton biomass nor any component thereof explained a significant portion of the residual variance in the TP–Chl *a* models of the ET sites.

Conclusions derived for the ET lakes were reinforced by the data assembled by Hanson and Peters (1984). Their data set encompasses a broad range of trophic conditions (TP varies from 4 to 200 $\mu g \cdot L^{-1}$) and crustacean zooplankton biomass

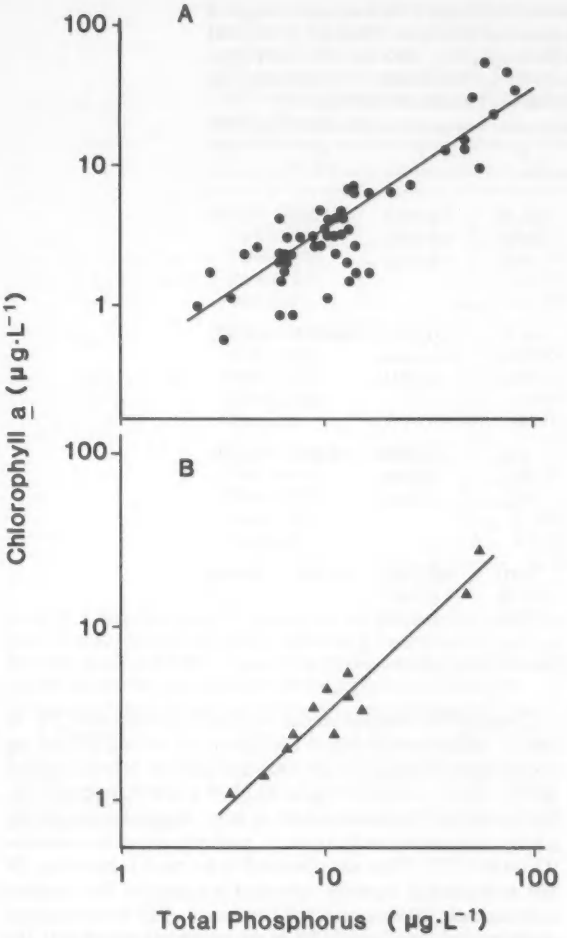

FIG. 2. TP–Chl *a* relationships for (A) all data from the ET sites (model: log Chl $a = -0.53 + 1.05 \log TP$, $F = 175.0$, $P < 0.0001$, $r^2 = 0.76$) and (B) seasonal means for the ET sites (model: log Chl $a = -0.56 + 1.09 \log TP$, $F = 136.7$, $P < 0.0001$, $r^2 = 0.93$). Note that the origins for the Chl *a* axes are slightly different in Fig. 2A and 2B.

(10.7–786 µg dw·L^{-1}). As with the ET data, zooplankton biomass was not a significant additional independent variable in a stepwise regression (Table 2).

To test the effect of zooplankton biomass within a lake, data from the three stations in Lake Memphremagog for 1981 and 1982 were used. Although a TP–Chl *a* model was highly significant for the within-lake data (log Chl $a = 0.715 \log TP - 0.79$, $F = 15.5$, $P = 0.0007$), only 41% of the total variance in Chl *a* was explained. There was, however, no significant multivariate model that incorporated either total zooplankton biomass (Table 2) or a component of the total for the within-lake data.

Zooplankton Community Structure

Tests for the effects of community structure on the TP–Chl *a* models were conducted using both the mean size of components of the zooplankton (i.e. cladocerans) and a general measure of the community based on the slope of the log abundance–log weight relationship. Mean cladoceran body size had a borderline significance when all the ET data were considered but was not significant for the seasonal mean values (Table 3). Within Lake Memphremagog, mean cladoceran body size was not significant in the multivariate test. Copepod and crustacean mean lengths were also not significant either for the Lake Memphremagog data or the ET data (all or seasonal means).

Differences in cladoceran body size in the ET lakes were due primarily to seasonal shifts in the relative abundance of *Daphnia* spp. (principally *D. galeata*) and *Bosmina longirostris*. These shifts were associated with variation in the TP–Chl *a* relationship during the ice-free season. Larger cladocerans tended to predominate when the TP–Chl *a* model underestimated Chl *a* and smaller cladocerans when it overestimated Chl *a*. The seasonal mean body sizes of cladocerans, however, varied little among sites (Table 1) and reflected an overall dominance by small (<1 mm) cladocerans. In this case, cladoceran body size had no effect on the TP–Chl *a* relationship.

In the 13 Indian River lakes studied by Mills and Schiavone (1982), there was a much larger variation in mean cladoceran body size (0.5–1.7 mm), with five lakes having mean sizes greater than 1 mm. When the effect of mean cladoceran size was tested with these data, it was significant at the 0.025 probability level (Table 3), and the effect was in the predicted direction as indicated by the negative partial regression coefficient associated with length (model: log Chl $a = 0.33 + 1.04 \log TP - 0.46L$).

Regressions for the log abundance–log weight relationships were in general highly significant (Table 4), and in only 4 of the 60 cases for the ET data were nonsignificant ($P > 0.05$) regressions found. Slope estimates tended to have large standard errors (Table 4) so that individual slopes generally were not significantly different from slopes obtained on other sampling dates. The slopes, however, were correlated with aspects of community structure. For example, the Spearman rank correlation between the slopes for the ET data and the ratio of macrozooplankton to microzooplankton biomass was highly significant ($r = 0.50$, $P < 0.0001$, $n = 60$). The slopes of the log abundance–log weight relationship were, therefore, used as a community structure (CS) index of the relative importance of the different size-classes of zooplankton.

When entered as an additional independent variable in the TP–Chl *a* models, the CS index was either significant at the 0.05 level or approached significance for the among- and within-lake data (Table 5). The partial regression coefficients associated with CS were negative in every case (Table 6), as predicted by the original hypothesis. Communities, therefore, dominated by small zooplankton have more Chl *a* per unit TP, while communities dominated by larger zooplankton have less.

The relative improvement provided by the stepwise multiple regression models involving the CS index was determined by taking the difference in the coefficients of determination (r^2) for the single (r_1^2) and multivariate (r_2^2) models and dividing by the proportion of the variance that was unexplained by the univariate model:

$$\Delta r^2 = (r_2^2 - r_1^2) \div (1 - r_2^2) \times 100\%.$$

This calculation yields a percent improvement in the sums of squares explained by the multivariate model. The improvement was only 4% in the case of all the ET data. For the seasonal mean data the absolute improvement in r^2 values was from 0.93 to

TABLE 3. Analyses of variance for models to predict Chl *a* from TP and mean body length of cladocerans (*L*) for (A) all data for the ice-free season of 1982 in the ET sites, (B) the 1982 ice-free season means for the ET sites, (C) Memphremagog only, 1981 and 1982, and (D) the Indian River lakes data set of Mills and Schiavone (1982). Coefficients of determination are from the univariate (r_1^2), TP alone, and multivariate (r_2^2) models for each case.

Source	df	SS	*F*	*P*	r_1^2	r_2^2
(A) All data						
Full model	2	8.341	104.48	<0.0001	0.782	0.798
Partial (TP)	1	8.341	208.95	<0.0001		
Partial (*L*)	1	0.160	4.01	0.0505		
Residual	53	2.116				
(B) Seasonal means						
Full model	2	1.660	61.51	<0.0001	0.932	0.932
Partial (TP)	1	1.659	122.99	<0.0001		
Partial (*L*)	1	0.000	0.00	0.9971		
Residual	9	0.121				
(C) Lake Memphremagog						
Full model	2	0.173	7.66	0.0032	0.414	0.422
Partial (TP)	1	0.173	15.32	0.0008		
Partial (*L*)	1	0.003	0.29	0.5952		
Residual	22	0.237				
(D) Indian River lakes						
Full model	2	3.774	73.07	<0.0001	0.892	0.936
Partial (TP)	1	0.843	32.65	0.0002		
Partial (*L*)	1	0.178	6.88	0.0255		
Residual	10	0.258				

TABLE 4. Summary of statistics for the regressions ($n = 60$) of log abundance versus log mean weight for all of the ET lake data.

Statistic	Median	Range
Slope	−0.658	−1.239 to −0.300
Standard error of the slope	0.164	0.101 to 0.334
Coefficient of determination	0.610	0.138 to 0.884
Probability > *F*	0.003	<0.0001 to 0.365

0.96 (Table 5). This represented a 43% improvement in the proportion of residual variance explained by the multivariate model. Addition of the CS index also accounted for 27% of the residual variance relative to the univariate model for the Lake Memphremagog data. In two of three cases, therefore, the CS index substantially reduced unexplained variance in the TP–Chl *a* models.

Discussion

The clearest result of this analysis was that zooplankton biomass had no influence on the TP–Chl *a* relationship. A similar conclusion was reached by Hoyer and Jones (1983), who measured the density of zooplankton along with TP and Chl *a* in 82 reservoirs. In their study, zooplankton densities were estimated using vertical net hauls that have a variable accuracy both in terms of filtration efficiency (M. L. Pace, unpubl. data) and in terms of the size-classes of zooplankton sampled (Likens and Gilbert 1970). In the present study the accuracy and precision of the zooplankton sampling methods were evaluated to ensure that representative estimates of both the microzooplankton and macrozooplankton were obtained. Despite this rigor in sampling, no component of the zooplankton biomass explained any of the residual variation in the TP–Chl *a* models.

Zooplankton biomass tended to covary strongly with TP. In the ET lakes the correlation coefficients (r) of log TP and log zooplankton biomass for the total data and the seasonal means were 0.70 ($P < 0.0001$) and 0.89 ($P < 0.0001$), respectively. Covariance of this nature results in large standard errors for the partial regression coefficients of multiple regression models (Gujarati 1978). This was observed in the models including TP and zooplankton biomass, where, for example, the standard error estimate for the regression coefficient of TP in the seasonal means model rose from 0.093 in the univariate model to 0.257 in the multivariate model. Covariance might then have obscured any differential effect on zooplankton (type II error). However, for the within-lake test, TP and zooplankton biomass were not correlated ($r = 0.249$, $P = 0.241$). This test was free of covariance, and zooplankton biomass still was not a significant variable (Table 2).

Covariance was also not a problem for the measures of community structure. Both the mean body size of cladocerans and the CS index varied independently of TP. For the ET data, the seasonal mean values represent the most appropriate among-systems test of the hypothesis that large zooplankton reduce the ratio of Chl *a* to TP, because TP–Chl *a* models are generally based on summer or "growing season" mean values. Mean cladoceran body size was not significant, but the CS measure was. Cladocerans were not large in the ET lakes, and in none of the 60 samples was the biomass dominated by a large daphnid such as *D. pulex*. These data then cannot be used to reject the hypothesis that large daphnids lower the Chl *a*:TP ratio (Shapiro and Wright 1984). The stepwise regression test of the data from the study by Mills and Schiavone (1982) where large daphnids were present lends support to the generality of the large daphnid effect. Data from all the samples in the ET lakes also suggest that larger cladocerans do lower the Chl *a*:TP ratio at various points of the summer season (Table 3). Further

TABLE 5. Analyses of variance for models to predict Chl *a* from TP and CS as measured by the slope of the regression of log abundance and log weight for the zooplankton community. Analyses are for models of (A) all data for the ice-free season of 1982 in the ET sites, (B) the 1982 ice-free season means for the ET sites, and (C) Lake Memphremagog, 1981 and 1982. Coefficients of determination are from the univariate (r_1^2), TP alone, and multivariate (r_2^2) models for each case.

Source	df	SS	*F*	*P*	r_1^2	r_2^2
(A) All data						
Full model	2	8.202	174.95	<0.0001	0.761	0.774
Partial (TP)	1	8.174	184.57	<0.0001		
Partial (CS)	1	0.142	3.21	0.0787		
Residual	54	2.533				
(B) Seasonal means						
Full model	2	1.705	101.11	<0.0001	0.932	0.957
Partial (TP)	1	1.513	179.39	<0.0001		
Partial (CS)	1	0.046	5.40	0.0452		
Residual	9	0.076				
(C) Lake Memphremagog						
Full model	2	0.197	9.97	0.0031	0.475	0.618
Partial (TP)	1	0.197	19.44	0.0009		
Partial (CS)	1	0.046	4.51	0.0551		
Residual	12	0.122				

TABLE 6. Stepwise multiple regression equations to predict Chl *a* from TP and community structure, where b_0 is the intercept term, b_1 is the slope associated with TP, b_2 is the slope associated with CS, and SE_1 and SE_2 are the standard errors of the regression coefficients.

Data set	b_0	b_1	SE_1	b_2	SE_2
All data	−0.72	1.06	0.078	−0.25	0.141
Seasonal means	−0.99	1.06	0.079	−0.67	0.290
Lake Memphremagog	−0.54	0.93	0.211	−0.32	0.148

among- and within-lake tests are required to establish the importance of cladocerans to TP–Chl *a* models. Data for this test should include lakes (or years within lakes) where large daphnids are present and lakes where they are absent.

The slope of the log abundance–log weight relationship (the CS index) measured the degree of dominance of microzooplankton or macrozooplankton (Fig. 1). When microzooplankton were dominant (CS highly negative), there was more Chl *a* per unit TP. For example, Central Basin in Lake Memphremagog, which had the most negative average CS value, had a Chl *a*:TP ratio of 0.46, while Brome, which had the least negative CS value, had a ratio of 0.24. This result was significant for both the ET and Lake Memphremagog data sets. The CS index should be interpreted cautiously. This index is derived from the slopes of regressions that contain substantial unexplained variance (Table 4). A greater range of values (~4×) was observed for the CS index than cladoceran length in either the ET lakes (range ~2×) or the Indian River lakes (range ~3×). It is often the case that variables observed over a greater range are significant in regression analyses, although there is no a priori reason why this should be so. Unfortunately, there are very few data in the literature where both the abundance and size of the total zooplankton community have been measured to test the generality of the results presented here. The significance of the CS index in this study does indicate that even in lakes where large daphnids are not abundant, the zooplankton community size structure appears to influence the TP–Chl *a* relationship.

Aspects of zooplankton community structure, however, do not appear to be generally useful for predicting algal biomass. Estimates of zooplankton biomass and community structure require an effort and expertise that far exceeds that required to measure Chl *a*. Monitoring zooplankton communities might be useful for within-lake studies during the course of eutrophication or restoration and in cases where TP–Chl *a* models provide poor predictions. In the situation where a large number of lakes are considered, as in regional lake management, zooplankton data will probably add little to predictive models.

The manipulation of aquatic food webs to enhance the abundance of large herbivorous zooplankton has been advocated as a means of improving water quality (Shapiro 1980a, 1980b). This study sought to test the generality that systems dominated by large zooplankton have lower ratios of Chl *a* to TP. The results support the hypothesis and indicate that the effect is not limited to lakes with large daphnid populations. Shifts in community structure between dominance by microzooplankton or macrozooplankton as measured by the CS index also influence the ratio. The effect of zooplankton, however, is weak. In each case where significance was observed, it was at or near the 0.05 criterion of acceptance. Given the weakness of the effect for a wide range of natural systems, dramatic reductions of algal biomass through food web manipulations may only be achievable by radical changes in a lake (such as the fish kill experiments by Stenson et al. (1978) and Shapiro and Wright (1984)). These changes are unlikely to be stable. To maintain water quality, the manipulation will need to be performed repeatedly. The costs associated with repeated manipulations should be weighed against more permanent solutions to water quality problems such as the reduction of nutrient inputs.

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Logging Impacts and Some Mechanisms that Determine the Size of Spring and Summer Populations of Coho Salmon Fry (*Oncorhynchus kisutch*) in Carnation Creek, British Columbia

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Scrivener, J. C., and B. C. Andersen. 1984. Logging impacts and some mechanisms that determine the size of spring and summer populations of coho salmon fry (*Oncorhynchus kisutch*) in Carnation Creek, British Columbia. Can. J. Fish. Aquat. Sci. 41: 1097–1105.

Natural patterns in emergence times, seaward movements, instream distributions, densities, and growth of coho salmon fry (*Oncorhynchus kisutch*) between March and September are contrasted with patterns observed during and after logging in the Carnation Creek watershed. After streamside logging in 1976–77, fry emerged up to 6 wk earlier and moved seaward more quickly than during years before logging. These observations are attributed to higher water temperatures during the winter and to emergence during a period of more frequent freshets. Increased fry movement from the stream could result in habitat being underutilized. In sections affected by intense streamside logging, the deposition of "fine" logging debris led to increased fry densities during the summers of 1977 and 1978. After major freshets in November 1978, which removed this fine debris and affected channel morphology in these sections, fry densities declined below those observed prior to logging. Growth rate of fry was inversely correlated with density in all stream sections. Growth rates, after correction for density, tended to be greater in all sections after the adjacent streamside was logged. Larger fry and more variable numbers of fry remained in the stream in September after logging than before logging. Their increased size is attributed to the longer growing season afforded by earlier emergence. This complex of interacting factors determines the number and size of fry in autumn and it can influence the production of smolts the following spring.

On compare les schèmes naturels de temps d'émergence, de migrations vers la mer, de répartitions en eau douce, de densités et de croissance des saumoneaux cohos (*Oncorhynchus kisutch*) (mars à septembre) et les schèmes observés pendant et après l'exploitation forestière dans le bassin versant du ruisseau Carnation. Après l'abattage des arbres le long du cours d'eau, en 1976 et 1977, les alevins sont apparus jusqu'à six semaines plus tôt et ont migré vers la mer plus rapidement que les années avant l'exploitation forestière. Ces faits sont imputables aux températures supérieures de l'eau, au cours de l'hiver, et à l'émergence pendant une période de crues plus fréquentes. Une émigration plus importante des alevins pourrait entraîner une sous-utilisation de l'habitat. Dans les parties touchées par une exploitation forestière intensive sur les rives, le dépôt de «menues» particules de bois a entraîné des densités accrues d'alevins pendant les étés de 1977 et de 1978. Après d'importantes crues en novembre 1978, lesquelles ont fait disparaître ces menus débris et altéré la morphologie du chenal dans les parties touchées, les densités des alevins ont chuté à des niveaux inférieurs à ceux observés avant l'exploitation. Le taux de croissance des alevins étit en corrélation inverse avec la densité enregistrée dans toutes les parties du ruisseau. Les taux de croissance, corrigés en fonction de la densité, étaient d'habitude supérieurs dans les toutes sections après l'exploitation forestière des rives adjacentes. En septembre, les alevins qui restaient dans le ruisseau après l'exploitation étaient plus gros et leur nombre variait beaucoup plus qu'avant l'exploitation. On impute cette taille accrue à une plus longue saison de croissance, attribuable à l'émergence prématurée. Cette série de facteurs conjugués déterminent le nombre et la taille des alevins à l'automne et peuvent influer sur la production de saumoneaux au printemps suivant.

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An understanding of the impacts of forest practices on salmonid resources is a primary concern of western North American resource managers. Sound resource decision making requires a thorough understanding of how these resources are integrated in watershed ecosystems. A 15-yr study was initiated in 1970 to examine the effects of logging on a small west coast watershed. Emphasis was placed on understanding watershed processes, because upslope and streamside activities can produce both negative and positive instream effects (Narver 1972; Cederholm et al. 1981; Murphy and Hall 1981).

This paper describes, and attempts to explain, what caused changes in the patterns of emergence, seaward movement, distribution, densities, size, and growth of coho salmon fry (*Oncorhynchus kisutch*) during spring and summer. These patterns have been described and discussed for more stable streams in Oregon (Chapman 1962, 1965; Moring and Lantz 1975), Washington (Cederholm et al. 1981), and British

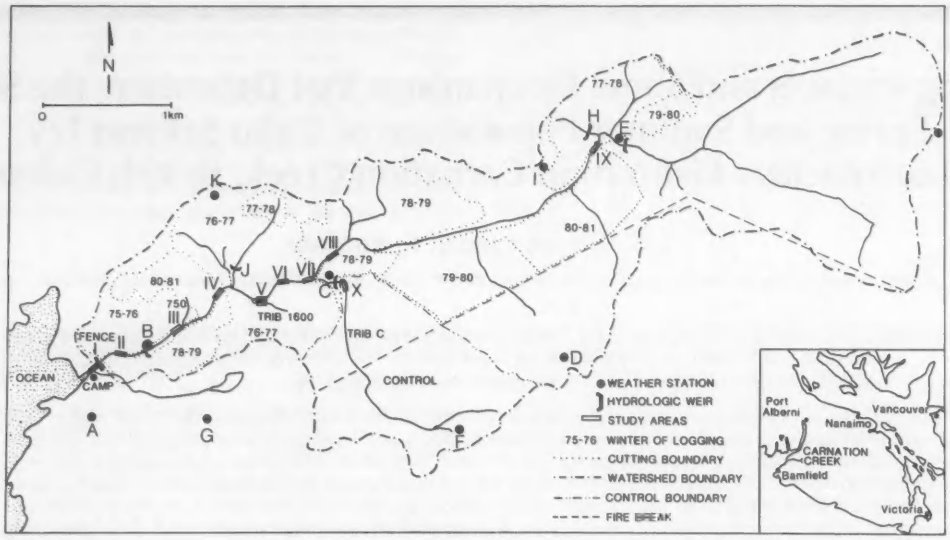

FIG. 1. Location of Carnation Creek and a detailed map of the watershed showing monitoring sites, stream study sections, and cutting boundaries.

Columbia (Mason 1975), but little long-term data are available from outercoast streams with very unstable flows.

Study Area and Logging Plans

Carnation Creek is a small salmon and trout nursery stream that flows into Barkley Sound on the west coast of Vancouver Island, B.C. (Fig. 1). The stream is about 7.8 km long and drains a watershed of ~10 km^2. It is located within the western hemlock biogeoclimatic subzone (Krajina 1969) and is typified by coarse, shallow soils, high annual precipitation (250–380 cm), little snowfall (<5% of precipitation), and widely varying flows (0.017–45 m^3/s). The major features of the watershed have been described in more detail elsewhere (Oswald 1973; Scrivener 1975; Bustard and Narver 1975).

Anadromous fish utlize the lower 3.1 km of stream encompassed by study sections I–VIII (Fig. 1). Chum salmon (*O. keta*) spawn mainly in section I, while coho salmon pass through the fence to spawn in areas represented by sections II–VIII. Study sections IX and X contain resident cutthroat trout (*Salmo clarki*). Small numbers of steelhead trout (*S. gairdneri*), sea-run cutthroat trout, and pink salmon (*O. gorbuscha*) spawn in the main channel. Coastrange sculpin (*Cottus aleuticus*) and prickly sculpin (*C. asper*) also occur in the lower main stream.

Timber harvesting began in the winter of 1975–76 with a small clearcut near the stream mouth (Fig. 1). This initiated three basic streamside treatments (undisturbed, intense, and careful) in the area occupied by salmon and steelhead trout.

Undisturbed Streamside Treatment

This area (sections II–IV) was logged as three openings during 1975–76 (23 ha), 1978–79 (19.4 ha), and 1980–81 (16 ha). This left the lower 1300 m of Carnation Creek with a small buffer strip or unlogged canyon area in which deciduous vegetation and a few merchantable trees remained.

Intense Streamside Treatment

The next 900 m segment of streamside (section V and VI) was logged in 1976–77 as two large clearcuts (61 and 34.4 ha). The streamside alders were girdled and injected with Tordon 22K herbicide. Later, during logging, merchantable timber was either felled away from the channel, or if leaning heavily, across it, and then yarded away from the stream to roadside landings.

Careful Streamside Treatment

Both sides of the next 900 m of streamside (section VIII) were logged as two clearcuts (63 and 13 ha) during 1978–79. Here, minor vegetation such as salmonberry was left; six merchantable trees that could not be jacked or cabled were felled across the channel and removed; alder was later felled and piled for burning during scarification.

Logging was begun in September–October and was ended in early May at these low elevation sites. High-lead cable systems were used. About 41% of the watershed had been logged upon completion of the last clearcut in May 1981. A more detailed description of the study sections is given elsewhere (Scrivener and Brownlee 1982; Toews and Moore 1982a).

Methods

Streamflow and water temperature were recorded at the hydrological weir at station B (Fig. 1). Continuous records of flow and stream temperature have been obtained since April 13, 1971, and July 16, 1970, respectively. Water levels were traced by Stevens A-35 and A-71 recorders. Automated processing techniques of Water Survey of Canada were used to extract and analyze the data (Ozga 1974). Similar computerized techniques were used to process water temperature charts that were recorded by a Lambrecht 256C thermograph. Mean daily water temperatures were then summed to produce total celsius temperature units (CTU).

During 1971 a temporary counting fence with three inclined plane traps was used to obtain daily counts of coho fry and smolts leaving the stream. This structure was 100 m upstream of the permanent fish counting fence, which was built in March 1972 (Fig. 1). The permanent fence consists of flow divider

screens and five fan traps capable of screening water at 6 m^3/s. At higher flows, known portions were subsampled. Length and weight data were obtained every 10 d for coho fry and more frequently for coho smolts. Fence construction and operations are explained more fully elsewhere (Lill and Sookachoff 1974; Hartman et al. 1982).

Large numbers of fry were caught at the counting fence a few days after they were first sighted by swimmers counting fish in the stream (Tschaplinski and Hartman 1983). These simultaneous observations indicated that early fence counts could be used to approximate the timing and magnitude of fry emergence. To compare annual variations in emergence and seaward movement of fry, five Julian dates were obtained each year form the daily fence counts. These were the dates of start, 10, 25, 75, and 90% of the fry run.

Similar counts were made of adult escapement during each autumn. Data of female escapement were used to indicate the timing of spawning and its distribution in time each year.

Lengths, weights, and population numbers used in this paper were also obtained from population surveys in the study sections (Fig. 1). Each year, three or more of these surveys were completed: one in late May, July, and September. Unfortunately, stream flow conditions did not permit completion of the surveys on exactly the same dates each year, but over the 13 yr reported here, most surveys were done within the 12-d periods of May 17–29, July 19–31, and September 13–25. Population surveys were initiated in sections II, III, VI, and VIII in 1970, in section IV in 1971, and in section V in September 1973. A lack of manpower prevented completion of the first survey in 1980.

Fish were collected by a combination of pole seining and electrofishing. The wetted areas of riffles and pools in each section were measured during each survey. In 1970 and 1971, coho population estimates were determined by mark–recapture methods. Later estimates were determined by removal method. Population survey methods are described elsewhere and all of the data on fish populations are available in data reports (Andersen 1978, 1981, 1983).

For analysis the data were divided either into two periods, 1970–76 (prelogging) and 1977–82 (post-intense treatment), or into three periods, 1970–76 (prelogging), 1977–78 (post-intense treatment to November 1978 freshet), and 1979–82 (post-freshet). The November 1978 storm was used to separate periods because this was the first major freshet after logging was begun. In some study sections streambanks were eroded, pools became shallower, gravel quality declined, and channelization occurred during this freshet (Schultz International Ltd. 1981; Toews and Moore 1982b). More recent freshets appear to be continuing these dramatic changes (Schultz International Ltd. 1981; Scrivener and Brownlee 1982).

Impacts from the logging activities can be masked by natural variability in numbers. Temporal and spatial variability are of most concern here, and in some other streams, spatial variability was the greater (Hall and Knight 1981). Temporal variability is assessed by the use of replicate years during the prelogging (7 y), the intense treatment (2 y), and the post-November 1978 freshet (4 y) periods. Spatial variability is reduced by combining data from some of the sections. Data from sections II + III and sections IV + VIII were combined and used as controls for sections V and VI, which were within the area of the intense treatment (Fig. 1). The usefulness of these controls was determined by examining the range of variability between sections during prelogging years. Populations in the study sections were expressed as numbers of fry per metre of stream instead of densities because this reduced variability that can be caused by differences in stream flow during each population estimate. The Mann–Whitney U-test is used for statistical comparisons of the data (Siegel 1956).

Fry lengths were used from the May and September population surveys to calculate growth rates. Measurements of fry from most study sections prior to September 1973 and from all sections after September 1973 permitted the calculation of growth rates within individual study sections. They were obtained from the formula

$$\text{Growth rate} = \ln(L_2/L_1)/(\text{Date}_2 - \text{Date}_1/30)$$

where L_1 is fork length on Date_1 and L_2 is fork length on Date_2.

Results

Seaward Movement of Fry

From 1971 to 1981, the number of fry moving out of Carnation Creek varied widely on a daily and annual basis (Fig. 2). Fry movement took place from early March through August, but the major period of seaward movement occurred in April and May. After 1976, the start of movement was generally earlier than before 1976, with the exception of 1971 and 1974. Also, 90% of the fry count at the fence occurred after April 15 in prelogging years 1971–76, but after 1976, 50–90% of the movement occurred prior to April 15 (Fig. 2).

Water temperature was positively correlated with the timing of emergence ($P < 0.0004$, Fig. 3). Thermal summation from November 1 was used because the maximum rate of female escapement and the first evidence of spawning was usually observed during the week that included this date. Between 60 and 90% of the total escapement had occurred by the end of the week that included November 1. Under controlled temperature regimes, the average observed requirement for emergence of coho fry is 1100 CTU (F. Velsen and D. Alderdice, Pacific Biological Station, nanaimo, B.C., pers. comm.). Both the dates of 1100 CTU accumulation and of 10% fry output were earlier in logging (1977–82) than in prelogging (1971–76) years (Mann–Whitney U-test, $P < 0.004$, Fig. 3).

Deviations from the correlation were large only during 1971 and 1974 (Fig. 3). During 1973, the year affecting 1974 emergence, the peak rate of escapement of female coho occurred earlier than in any other year (October 14–20, or 15 d prior to November 1). Accurate escapement counts were not obtained during autumn 1970, but adults were not seen and spawning was not evident until November 12 (11 d after November 1). To determine the influence of a varying period of spawning on the correlation, residuals were plotted against the differences between the Julian date of November 1 and the date of 70% escapement of females. During 1973, the date during the week of maximum escapement, October 17, was used because 50% of females passed through the fence prior to this date and because low water between October 28 and November 10 prevented passage of adults through the fence. A positive correlation was observed ($r = 0.68$, $P < 0.015$). Thermal summation from these dates produced a better correlation when the Julian date of 1100 CTU accumulation was regressed against the Julian date of the first decile of fry output ($r = 0.93$, $P < 0.0001$, $Y = 0.521X + 27.13$ vs. Fig. 3).

Many peaks in daily fence counts appeared to be associated with storms and high flows, but fry numbers were often not proportional to flow magnitude (Fig. 2). However, the number

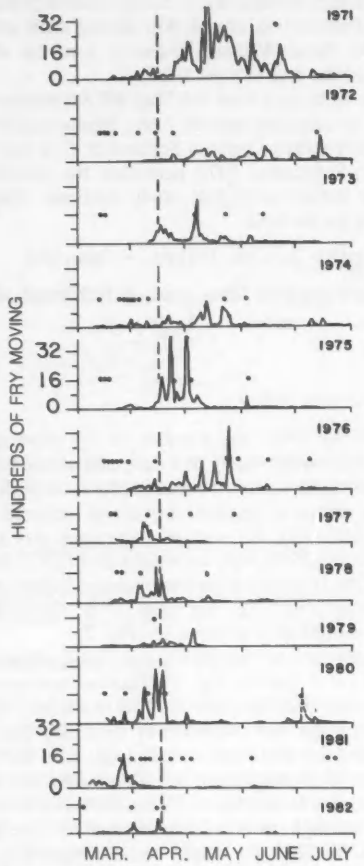

FIG. 2. Daily movement of coho fry through the Carnation Creek counting fence in 1971–82. Dots indicate the dates of freshets where peak flow exceeded 1.8 m^3/s. April 15 is emphasized with a broken line through all years.

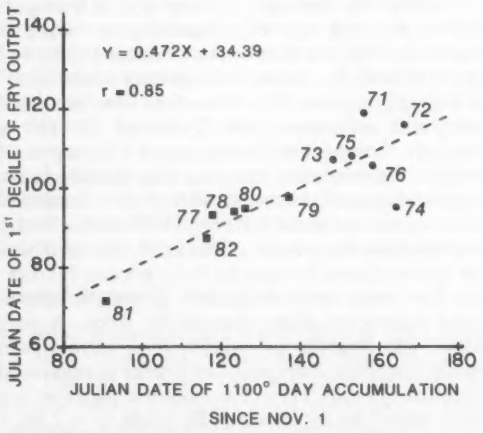

FIG. 3. Relationship between winter water temperatures (degree-days) at B weir and the first decile (10%) date of coho fry emergence and emigration in Carnation Creek. ●, prelogging years; ■, years during logging.

of days during which half of the annual movement of fry took place was inversely correlated with flow ($P < 0.0002$, Fig. 4). The number of days between the first decile (10%) and the third quartile (75%) of movement was also inversely related to the mean flow between these dates ($r = -0.67$, $P < 0.017$, $Y = -0.04X + 50.75$). Residuals from these relationships were not correlated with the length of the escapement period during the previous autumn ($r \leq 0.28$, $P > 0.35$). During springs with intense storms and high streamflows, the bulk of the fry moved over shorter periods of time than during springs with fewer storms and lower flows. With the exception of 1975, the bulk of fry movement occurred more quickly during years of logging 1977–82 (Mann–Whitney U-test, $P = 0.021$, Fig. 4; $P = 0.013$ for time between the first decile and third quartile).

Summer Numbers and Size of Fry

Between 1971 and 1982, the range of fry numbers in the stream was large, but the scenarios of most years followed one of three patterns (Table 1). In 1975, 1977, 1981, and 1982, fry numbers were low at the time of the first population estimate. This occurred because the number of emerging fry was low, because initial movement was high, or because both factors acted in concert (Fig. 2; Table 1). Numbers then declined slowly over the summer (Table 1). During 1976, 1978, 1979, and 1980, large numbers of fry remained in the stream throughout the summer and numbers declined gradually. During 1972 and 1973, substantial losses occurred between population estimates. These losses coincided with movement during freshets and occurred immediately after the May 18 population estimate in 1973 (May 24, June 7, Fig. 2) but occurred later in 1972 during July 14 (Fig. 2) and August freshets (Table 1).

The range in number of fry had been greatly reduced by September (Table 1). This was particularly true prior to 1979, but during later years the range increased by a factor of 3.5. During 1980, the numbers of fry remained very large despite considerable movement (Fig. 2). This was the result of a very large escapement of females during the previous autumn (176 in 1979; range 51–81 during 1971–78) and subsequently, of a large emergence of fry. During 1981 and 1982, fry numbers and fry movement were very low because of low emergence (Table 1; Fig. 2).

Mean lengths of fry were significantly greater after logging was begun (Table 2). This was apparent during both May–June and September population surveys. Fry were larger than the prelogging means during 1975 and larger than the postlogging means during 1981 and 1982 (Table 2) when numbers were low (Table 1). Fry tended to be smaller than these means during years when numbers were high for most of the summer (1972, 1976, and 1980).

Distribution and Growth in the Study Sections

The densities of fry in the study sections were highly variable among population estimates and among years. During late July, densities in study sections VI and VIII ranged from 4.8/m^2 in 1971 to 0.23/m^2 in 1981 (Fig. 5). In spite of this variability, three basic patterns were apparent. With the exception of 1971, densities in section VI were 1.2–1.9 times greater than those in section VIII for prelogging years (1970–76). They were 2.2 and 2.1 times greater in 1977 and 1978, respectively. Between 1979 and 1982, they ranged from 0.3 to 1.1 times those of section VIII (Fig. 5). The probabilities that the higher ratios in 1977–78 and that the lower ratios in 1979–82 were due to chance were

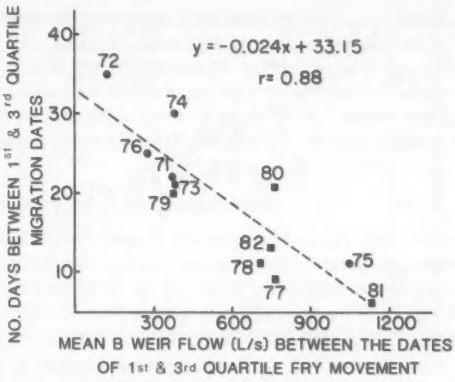

FIG. 4. Relationship between mean stream flow and the number of days between the first quartile (25%) and the third quartile (75%) of coho fry counts through the fence at Carnation Creek. ●, prelogging years; ■, years during logging.

0.056 and 0.003, respectively (Mann–Whitney *U*-test). Therefore, in relation to section VIII, densities in study section VI were higher immediately after the intense logging treatment, but subsequently, they were lower after the November 1978 freshet.

The ratios of study sections VI and IV densities were also lower after 1978 (Mann–Whitney *U*-test, $P = 0.024$, Fig. 5). Therefore, after the November 1978 freshet, densities in section VI were lower in relation to the sections immediately above and below the intense logging treatment.

The September data provided a more complete picture of these three basic phases within the sections that received the intense treatment (Fig. 6). Spatial variation during prelogging years ranged from 0.7 fry/m of stream (1970) to 2.6 fry/m

TABLE 1. Population estimates of coho fry and their 95% confidence intervals in the lower 3070 m of Carnation Creek. Underlined estimates differ slightly from those of Andersen (1978, 1980, 1983) because they were adjusted to the same weekly period each year by adding or substracting the number of fry counted at the fence during the days of adjustment.

Year	Population estimate period: Late May–early June	Late July	Late September
1971	—	—	11 842±2260
1972	33 556±3860	15 578±2099	9 223±1130
1973	18 357±3984	10 776±1098	9 071±1225
1974	17 580±973	14 589±385	12 460±455
1975	14 605±3427	14 092±413	11 482±309
1976	46 662±1054	19 173±470	12 327±634
September mean			11 088
1977	15 767±1098	14 338±328	10 602±395
1978	26 148±368	23 120±271	10 927±212
1979	26 437±608	19 702±351	13 227±325
1980	—	25 756±858	20 953±350
1981	6 743±619	6 995±452	6 088±243[a]
1982	8 648±610	8 890±180	7 337±166
September mean			11 522

[a]Data from section II excluded in this estimate because storms prevented its completion until the 2nd wk in October.

TABLE 2. Mean lengths of coho fry in Carnation Creek during late May and September population estimates both before and during logging activity ($\bar{x} \pm 95\%$ CL). Statistical comparisons of prelogging and postlogging means were made with Students' *t*-test. Since a May 1980 population estimate was not done, mean coho fry length at the counting fence was used.

Year	Date		Mean length ± CL	Date	Mean length ± CL
1970	—		—	Sept. 15	52.4±0.9
1971	May 8		39.7±0.6	Sept. 15	47.9±0.5
1972	May 24		38.1±0.4	Sept. 12	46.1±1.6
1973	May 18		39.5±0.3	Sept. 12	50.6±0.5
1974	May 21		40.1±0.2	Sept. 18	49.4±0.5
1975	May 21		40.1±0.9	Sept. 18	54.3±1.0
1976	May 19		39.8±0.4	Sept. 22	49.2±0.9
Prelogging mean			39.5		50.0
1977	May 17		41.8±0.9	Sept. 25	59.9±1.0
1978	June 4		44.1±0.6	Sept. 25	57.1±0.9
1979	June 4		45.2±0.7	Sept. 19	58.0±0.7
1980	June 2	Fence:	39.8±0.8	Sept. 17	51.7±0.3
1981	May 21		46.2±0.9	Sept. 16	66.5±1.1
1982	May 26		44.8±0.7	Sept. 22	65.6±1.2
Postlogging mean			43.7		59.8
t			3.98**		3.93**

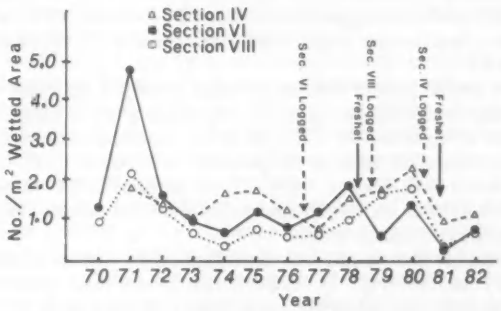

FIG. 5. Late July densities of coho fry in Carnation Creek study sections IV, VI, and VIII. The winters of adjacent logging and major freshets are indicated.

(1974). During 1977, variability increased to 5.3/m and a large portion of the fry population was resident within the area of intense treatment (Fig. 6). Spatial variation declined to 1.9 fish/m during 1978, but fry numbers were still higher than those of the other two areas. When fry per metre within the intense treatment are expressed as a percentage of those from the other two areas, values ranged from 57 to 191% during prelogging years and from 128 to 416% during 1977 and 1978. The probabilities of observing these higher values within the intense treatment when compared with sections II + III and sections IV + VIII were 0.056 and 0.028, respectively (Mann–Whitney *U*-test). Spatial variability increased again to 5.2 fish/m after the November 1978 freshet, but now the component of the population within the intense treatment was relatively small (Fig. 6). Between 1979 and 1982, values ranged from 26 to 111% of numbers per metre in the other two areas. Probabilities of 0.055 and 0.006 were obtained for observing these percentages that were smaller than those of prelogging in sections II + III and IV + VIII, respectively (Mann–Whitney

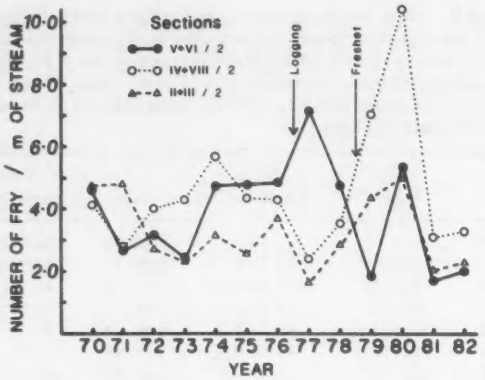

FIG. 6. Relative abundance of fry through time among study sections II + III, IV + VIII, and V + VI in Carnation Creek during late September. Values were calculated for 1973 section VIII and for 1981 section II. The proportion of the total population (Table 1) found in the study section during other surveys was regressed against the population estimate. Data were used from all other population surveys of the year and from those of the two following and preceding years (14 points, $r = 0.87$, $P = 0.0001$).

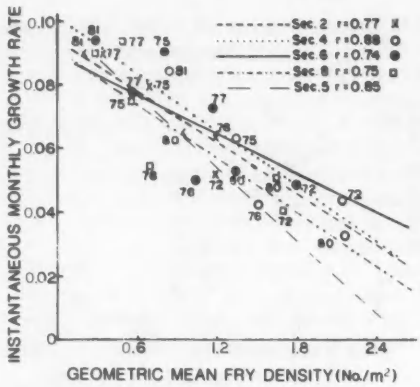

FIG. 7. Relationship between geometric mean coho fry density and instantaneous 30-d growth rate in Carnation Creek study sections II, IV, V, VI, and VIII. Data points for years discussed in the text are plotted.

U-test). Within the intense treatment, numbers per metre increased significantly after logging and then dropped significantly below prelogging levels after the November 1978, and subsequent freshets began making changes to study sections V and VI.

A similar pattern was not obtained when fry per metre of stream in sections II + III were expressed as a percentage of those in sections IV + VIII (Fig. 6). Significant differences were not found between the periods 1970–76 and 1977–78, between 1970–76 and 1979–82, or between 1970–78 and 1979–82 after the careful streamside treatment occurred adjacent to study section VIII.

Summer growth rate was dependent on fry density in each study section (Fig. 7). Growth rate of fry was inversely correlated with geometric mean density in sections II ($P < 0.002$), III ($r = -0.78$, $P = 0.003$), IV ($P < 0.001$), V ($P < 0.004$), VI ($P < 0.003$), and VIII ($P = 0.003$). Data from section III were not illustrated in order to preserve the readability of Fig. 7. Geometric mean was used because during spring and summer, numbers and densities from populations of coho fry tended to decline as a geometrical rather than a linear progression (Table 1; Chapman 1965). Analysis of variance of regression coefficients (growth rate on density) indicated no difference between sections in slope or intercept when data from all years were used ($P > 0.53$). Therefore, the growth rates could be combined for all sections. A covariance analysis with sections and logging as grouping factors, and with density as the covariate, indicated a significant relationship between growth and density ($F_{1,59} = 94.26$***), no difference between sections after adjustment for density ($F_{5,59} = 0.85$), a significant increase in growth rate after logging ($F_{1,59} = 10.89$**), and no interaction between groups ($F_{5,59} = 0.66$). The pre- and post-logging periods were slightly different for each study section because the area adjacent to each section was logged during different years (Fig. 1).

When growth rate was adjusted for density, postlogging means were greater than prelogging means at all six stream sections, but this increase was substantial only for sections V and VIII. The smallest change was observed in section VI (0.0677 vs. 0.0714), while the largest change occurred in section VIII (0.0577 vs. 0.0751). When the covariance analysis was repeated using data from sections II, III, IV, and VI, growth rate was still dependent on the covariate density ($F_{1,41} = 57.74$***), but the increase in growth rate after logging was barely statistically significant ($F_{1,41} = 4.20$, $P = 0.048$). The increase was not statistically significant when any combination of three of these sections was used in a covariance analysis (sections III, IV, and VI, $F_{1,30} = 2.77$, $P = 0.110$; sections II, III, and VI, $F_{1,31} = 4.08$, $P = 0.052$). Therefore, the increase in growth rate was not statistically significant in the undisturbed streamside treatment (sections II, III, and IV, $F_{1,30} = 3.66$, $P = 0.066$). When data from the areas of intense and careful streamside treatments (sections V, VI, and VIII) were used in a covariance analysis, growth rate was dependent on density ($F_{1,28} = 37.22$***) and the increase in growth rate after logging was statistically significant ($F_{1,28} = 7.03$*, $P = 0.013$). Therefore, even though postlogging growth rates tended to be greater in all study sections, it was mainly the data from sections V and VIII that produced statistical significance. Sections V and VIII also had the lowest overall growth rates (Fig. 7).

Since density and growth rates were inversely related and since a wide range of densities were obtained, growth rates varied from year to year (Fig. 7). During 1975, 1977, and 1981, growth rates (Fig. 7) were higher when densities and fry numbers in the stream were low (Table 1). In years such as 1972, 1976, and 1980, growth rates tended to be low when numbers and densities were high.

Discussion

Our results indicated that a complex of factors influenced the numbers of coho salmon fry and their movement, distribution, and growth during spring and summer. The interaction of these factors ultimately determined the number and size of fry in September. Some of them were affected by logging. One factor was the timing of emergence, which was related to water temperature in winter. This determined the length of the growing season. Another factor was discharge as it affected movement of fry out of the stream. The distribution of fry in the study sections was influenced by logging in the area of intense

treatment. Growth rates were dependent mainly on density, but they were also influenced by logging. Length of growing season and growth rate determined the size of fry in autumn. Since an increase in size tended to increase the probability of overwinter survival (Hartman et al. 1984), these factors can have an important influence on the stream's production of coho smolts.

Emergence and Water Temperatures

From 1977 onward, fry appeared at the fence earlier and usually stopped moving sooner than in prelogging years. This did not appear to be the result of earlier egg deposition or stress-related early emergence (Hartman et al. 1982). Egg-to-fry development was more rapid and related to the higher winter water temperatures observed since 1975–76. Similar results were obtained after logging of Needle Branch Creek, Oregon (Ringler and Hall 1975). The confounded effects on stream temperature of logging and climatic variability were separated through the use of an empirical model that predicted stream temperature from air temperature, solar radiation, and stream discharge (Holtby and Newcombe 1982). Observed water temperatures were higher than those predicted by the model during all five winters of logging (1976–77 to 1980–81) and significantly higher statistically during two of these years (Hartman et al. 1984). Therefore, the observed increase was influenced both by logging and by warmer weather.

In Carnation Creek the warmer temperatures in winter were probably the result of a combination of factors. First, warmer air temperatures and increased solar radiation were observed between 1976–77 and 1980–81 (Holtby and Newcombe 1982). Water temperatures in winter were also strongly correlated with air temperatures elsewhere in Canada (Cluis 1972) and in Alaska (Sheridan 1961). At Carnation Creek this relationship is rarely weakened by influences from snowmelt or ice cover as in Alaskan streams (Sheridan 1961). The relationship and solar radiation inputs would become more pronounced when the moderating influence of a forest canopy was removed. This was observed in the Alsea study where both mean temperatures and diel fluctuations of stream and intragravel water increased in relation to the extent of logging (Ringler and Hall 1975).

Groundwater influences also affected stream temperatures at Carnation Creek. Groundwater inputs produced intragravel temperatures that rose an average of 1–1.5° above those of the stream during each November and that fell below them after March (Hartman and Leahy 1983). At some sites the differences in temperature were as great as 5.9° during the winter. This pattern was apparent during both early morning and late afternoon measurements and therefore it was not an artifact of diel fluctuations that were observed elsewhere (Ringler and Hall 1975). This temperature gradient was also not a result of reduced exchange of surface with intragravel water caused by deposition of fine sediment (Ringler and Hall 1975). The pattern was observed at many locations along the length of the stream (Hartman and Leahy 1983), and prior to 1980, significant accumulations of particles smaller than coarse sand have not been observed above the area of tidal influence in Carnation Creek (Scrivener and Brownlee 1982). We speculate that higher temperatures in groundwater have occurred because more radiant energy has been stored over the summer in the soils of burned clearcuts. It has subsequently been transferred to groundwater by autumn and early winter precipitation passing through the soil (Hartman et al. 1984). Evidence from two areas support this hypothesis. First, significant differences between model-predicted and observed stream temperatures in winter did not occur until 12% of the watershed had been logged and burned. Observed stream temperatures had also returned to near predicted values during the fifth and last year of logging (Hartman et al. 1984), when only 5% of the watershed was logged and when the large areas that were initially clearcut had reestablished a ground cover (King and Oswald 1982). Second, the strong correlation between the temperatures of stream and of intragravel water throughout the year was different during late November and December (Hartman and Leahy 1983). Intragravel water was warmer in relation to the stream during the period when this heat transfer is hypothesized to occur.

Movement of Fry

The maintenance of adequate fry stocks in the stream could be adversely affected by shifting the period of emergence into the stormy March–early April period. The early rapid loss of fry during freshets apparently resulted in the stream being underutilized in 1981 and 1982. It has been demonstrated that coho fry in Carnation Creek tend to move seaward mainly during freshets (Hartman et al. 1982) and that fry emerging earlier in the spring have an increased probability of encountering high flows (Hartman et al. 1984). Results here indicated that during years of intense storms and high-stream flow, the bulk of fry loss to the stream occurred earlier than in years with few storms and lower flows. The flow conditions at the time of emergence appear to have an important impact on how quickly fry numbers are reduced during spring.

In 1975, 1977, and 1981, most losses occurred so quickly that it is unlikely that density-dependent displacement influences of territoriality and aggression described elsewhere (Chapman 1962, 1966; Mason and Chapman 1965) could have had time to influence movement. Later, or during other years, aggressive interaction probably served to force fry into less favorable habitat in the stream, but these were usually displaced downstream and through the counting fence during periods of freshet (Hartman et al. 1982). Displacement during periods of low light intensity or lunar phases, as suggested by Mason (1975) for Lymn Creek, was not observed at Carnation Creek (Hartman et al. 1982).

In Carnation Creek during 1981 and 1982 an understocking of coho fry was attributed to a reduction in egg-to-fry survival (Scrivener and Brownlee 1982) and to high flows that occurred during emergence. Understocking was also observed for the Clearwater River, Washington (Cederholm et al. 1981). It occurred because of low adult escapement and reduced egg-to-fry survival. The latter was attributed to an accumulation of fines that were washed from heavily used logging roads (Cederholm et al. 1981).

Summer Distribution of Fry

Changes in fry densities within study sections subjected to the intense logging treatment coincided with observed alterations in organic debris distribution and in channel morphology. After the intense logging treatment, the volume and stability of large organic debris in sections V and VI were reduced (Toews and Moore 1982a). The debris consisted of more individual and much smaller pieces. It had remained relatively unchanged in other sections (Toews and Moore 1982b). Photos also indicated that substantial quantities of fine debris such as branches and deciduous vegetation had been introduced to sections V and VI. Coincident with these additions of debris, 1977 summer fry

densities doubled in relation to other sections. Fry densities were greater probably because the additions of scattered fine debris divided the stream into physically more complex habitat that supported greater numbers. This impact was still apparent during the summer of 1978, after a winter of few freshets.

Major reductions in the volume of debris and changes in channel morphology occurred after the first large freshet during logging in November 1978 (Toews and Moore 1982b; Schultz International Ltd. 1981). Streambanks were eroded, pools were shallower, and the channel was straightened in these study sections. Fine debris had been redistributed into piles at a number of locations by 1979, and these were reduced in volume by a factor of 10 between 1979 and 1981 (Schultz International Ltd. 1981). Coincident with these changes, fry densities in relation to the other study sections were reduced to about a half of prelogging values. Channel and streambed changes appeared to be continuing in 1980–81 (Scrivener and Brownlee 1982). Stream channelization in Washington State reduced coho densities in some creeks (Cederholm and Koski 1977), but this did not necessarily result in a reduction in biomass (Chapman and Knudsen 1980).

Summer Growth and Size of Fry

Densities appeared to have a large influence on growth rates in all study sections. After adjustment for density, postlogging summer growth rates were significantly greater only in sections V and VIII. They also had the lowest observed prelogging rates and received the intense or careful streamside treatment. Despite this modest influence of logging on summer growth rates, fry were much larger in September. These observations suggested that it was the earlier emergence during logging and the consequent longer growing season that had the greater impact on September sizes of fry. Their size by the end of May had the greatest impact on size in September when simulation techniques were used to examine growth (Hartman et al. 1984). Fry were larger after 1976 because of a combination of longer growing period and higher spring growth rates. The more rapid reduction in fry numbers in logging years probably contributed to these higher spring growth rates.

General Discussion and Summary

The data presented in this paper can contribute to the long-standing discussion on the relative importance of changes in growth rate and in mortality/emigration rate with density as they affect populations of juvenile coho (Chapman 1962, 1965, 1966; Mason 1975, 1976; Mason and Chapman 1965). In spring and summer, the physical environment sets boundaries on the population and does not react to the biological aspects. Actual density is then regulated by a space–food mechanism involving social interaction and displacement of fry that "drift" downstream (Chapman 1966). In Carnation Creek, fry may emerge early or during a spring with very unstable flows. During these years, the physical environment can legislate such low numbers that social interaction makes only minor adjustments to these numbers during the remainder of the spring and summer (1975, 1981, 1982).

The spatial requirements of coho also seem to be very flexible. Supplemental feeding maintained densities of 1.2–4.5 times those in control sections of Sandy Creek (Mason 1976). Growth rates in the control sections were inversely related to population density. In Carnation Creek, introduction of fine logging debris produced densities 1.4–2.9 times those of prelogging years. This occurred at a time when aquatic food resources were either unchanged or reduced (Culp and Davies 1983). This manipulation of density made the strong inverse correlation between density and growth more apparent. The greater densities and growth did not lead to an increase in smolt production in Sandy Creek (Mason 1976), but an increase was observed at Carnation Creek (Andersen 1983). In the former case, it did not occur because shelter habitat that limits densities during the winter had remained unchanged (Mason 1976). In the latter case, this occurred because winters were more benign and because most juveniles were larger and now underwent smoltification after 1 yr in the stream (Hartman et al. 1984).

Results from this study support recent hypotheses of two groups of logging-related influences that have appeared in the literature (Chapman and Knudsen 1980; Murphy and Hall 1981; Murphy et al. 1981; Martin et al. 1981). Opening of the canopy over a stream tends to improve the rate of energy transfer in the biological community. As a result, microbial respiration, primary production, densities of benthos, drift of food organisms, and densities of predators including salmonids can all increase. These impacts are most apparent when heavily shaded, oligotrophic, higher graident, boulder substrate streams are exposed to sunlight, and they become less apparent in larger, more open, lower gradient streams. The impacts are short-lived (1–15 yr after logging) and disappear as the driving forces of sunlight on the stream and the increased free nutrients are reduced by the second growth forest.

The second group of logging impacts are related to habitat changes and their influence is usually negative. These impacts are usually of longer duration and can still be apparent 35–50 yr after logging. They are most pronounced in low-gradient streams where mechanical energy may not be adequate to transport introduced fine particles, which then accumulate in the gravel. Habitat changes are also common in streams that are dependent on woody debris to provide, first, channel and bed stability and, second, stable cover or surfaces on which stream biota depend. It probably takes more than 60 yr before new large organic debris begins falling into the stream to replace losses due to logging. In higher gradient streams or rivers, large boulders help to perform these stabilizing functions. This division of influences into energy and structural habitat related impacts aids in our understanding of the mixture of positive and negative influences observed at Carnation Creek.

The need to understand the processes and mechanisms affecting size and number of fry observed during autumn can be emphasized by presenting various annual scenarios observed at Carnation Creek. During the prelogging years 1972 and 1976, fry emerged late in the spring after the freshets. This resulted in a shorter growing period and a prolonged period of fry movement through the counting fence. The high densities produced low growth rates and smaller than average fry in September. Freshets in late July and August 1972 produced high losses through the counting fence and thereby both small size and numbers in September. During 1975, fry emerged earlier because of warmer winter water temperatures and were exposed to freshets that rapidly reduced numbers. Few losses later in the summer left below average prelogging densities, but the resulting higher growth rates and longer growing period produced the largest prelogging fry in September. During logging years 1977 and 1981, fry emerged very early after warmwater temperatures, but were immediately exposed to freshets. Numbers of fry were rapidly reduced. The low

densities induced high growth rates that, coupled with the longer growing period, produced the largest fry in September. During 1980, emergence of very large numbers of fry was obtained from double the average adult escapement. This left large numbers in the stream even after substantial loss over a long period. As a result, numbers in September were very high. Growth rates were very low, but the long growing period produced fish that were within the prelogging size range.

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Early Life History of Redfish (*Sebastes* spp.) on Flemish Cap

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Anderson, J. T. 1984. Early life history of redfish (*Sebastes* spp.) on Flemish Cap. Can. J. Fish. Aquat. Sci. 41: 1106–1116.

Studies were carried out on Flemish Cap, 1978–82, to assess fish spawning cycles, the distribution, abundance, and growth of early life stages, and their relationship to environmental factors. Redfish larvae (*Sebastes* spp.) were the most abundant fish larvae found on Flemish Cap. Redfish began releasing larvae during March, reaching an abrupt peak in late April. Larval abundances of 733 larvae·m^2 were observed during the last week of April 1979, with highest sampled larval abundance for the study area being 6.8×10^{12} larvae. Redfish larvae first appeared in the southwest corner of Flemish Cap and within 3 wk were found in waters throughout the area over depths >200 m. In July the survivors were concentrated over the Cap supporting the concept that Flemish Cap redfish constitute a distinct group. Larval growth for redfish was exponential through the periods sampled. However, in 1981, growth rate ranged from 0.40 to 1.66%·d^1. While larval growth was significantly correlated with average surface water temperature, high temperatures appeared to reduce larval growth. Slow growth during warm years appeared to be related to increased larval mortalities.

De 1978 à 1982, on a mené des études dans la région du Bonnet Flamand afin d'évaluer les cycles de fraie du poisson, la répartition, l'abondance et la croissance des individus aux premiers stades larvaires, et la relation entre ces variables et les facteurs environnementaux. Les larves de sébaste (*Sebastes* spp.) étaient les plus abondantes, apparaissant en mars et atteignat une concentration maximale à la fin d'avril. Pendant la dernière semaine d'avril 1979, l'abondance des larves s'élevait à 733 larves·m^2 et la plus haute abondance échantillonnée dans la zone étudiée totalisait $6,9 \times 10^{12}$ larves. Les larves de sébaste ont été signalées pour la première fois dans la partie sud-ouest du Bonnet Flamand et, 3 sem plus tard, étaient présentes dans toute la zone d'une profondeur supérieure à 200 m. En juillet, les survivantes étaient concentrées dans la région du Bonnet, fait qui appuie le concept selon lequel le sébaste du Bonnet Flamand représente un groupe distinct. La croissance larvaire a été exponentielle pendant toutes les périodes échantillonnées. Toutefois, en 1981, le taux de croissance variait de 0,40 à 1,66 %·$jour^1$. Quoique la croissance larvaire était en corrélation directe significative avec la température moyenne des eaux superficielles, les températures élevées semblaient réduire la croissance. Et une telle croissance ralentie pendant les années chaudes paraissait reliée à une mortalité accrue des larves.

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Flemish Cap (47°N, 45°W) has been the subject of an international fisheries investigation in recent years. The basis of this research, and indeed of existing fisheries theory, is that recruitment in commercial fishes is determined during the first year of life (Gulland 1965; May 1974; Cushing 1975). Studies have been carried out in many areas of the world in an attempt to determine factors affecting survival of fish eggs and larvae (e.g. May 1974; Lasker and Sherman 1981). Following the assumption that environmental factors play a key role in larval fish survival, Flemish Cap was identified as a promising area in which to carry out a study to assess factors controlling fish production (Anonymous 1975; Templeman 1976).

Flemish Cap is an unique oceanic area lying to the east of the Newfoundland Grand Banks, separated from it by the 1000 m deep Flemish Pass (Fig. 1). Biologically the area is distinct from the adjacent Grand Banks. Only three groups of commercial fish are present: Atlantic cod (*Gadus morhua*), Atlantic redfish (*Sebastes* spp.), and, of minor importance, American plaice (*Hippoglossoides platessoides*). There is no pelagic fish community similar to that of the adjacent Grand Banks. Capelin (*Mallotus villosus*) and sandlance (*Ammodytes* spp.), historically found in abundance on the Grand Banks, are rarely observed on Flemish Cap (Templeman 1976). In addition, Flemish Cap cod form a distinct population (Templeman 1974; Cross and Payne 1978; Lear et al. 1979).

Oceanographically, the area is also distinct. The bank is bounded by two major oceanic current systems: the cold, low-salinity Labrador Current flowing south through Flemish Pass and east along the northern flank and the comparatively warm, high-salinity North Atlantic Current flowing from west to east along the southern flank of the Cap (Fig. 1). A weak anticyclonic gyre overlies the central portion of Flemish Cap with Broad areas of mixed water occurring in intermediate areas (C. Ross, Atlantic Oceanographic Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.). Water temperatures seldom fall below 3°C, which is warm compared with the adjacent Grand Banks.

Ichthyoplankton and oceanographic surveys were carried out on Flemish Cap at different times during 1978–82 to determine the nature of egg and larval distributions, growth, mortality, and how these might change between years. Reported here are the results for larval redfish, by far the most abundant larval fish found on Flemish Cap. The timing of larval release, changes in

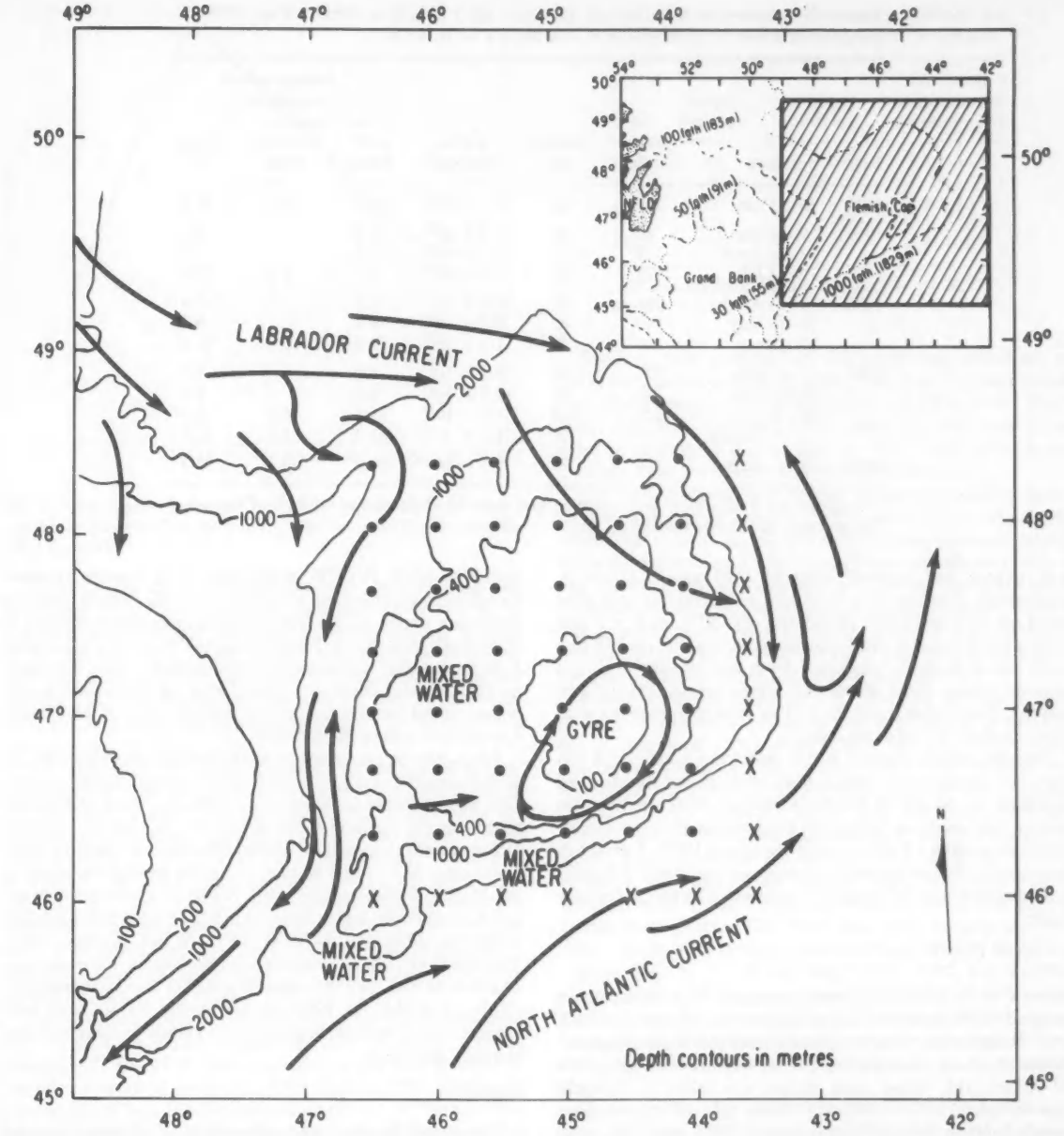

FIG. 1. Flemish Cap bank, lying east of the Newfoundland Grand Banks, showing the main oceanographic features of this region, and the study sampling grid.

distributions and abundances through the spring–early summer period, and characteristics of larval redfish growth and mortality are reported.

Materials and Methods

Twelve ichthyoplankton surveys were carried out during 1978–82 on Flemish Cap. Samples were taken using a survey grid at 20 nautical miles (37 km) station spacing, ranging from 20 to 56 stations per survey, which each lasted 3–7 d (Table 1). At each station, ichthyoplankton and physical oceanographic sampling were carried out. During these four years, sampling dates ranged from the last week of March in 1979 to the first week of August in 1981 and 1982.

Ichthyoplankton sampling was done using 61 cm Bongo nets towed at $\sim 1.5\ m \cdot s^{-1}$ (3.0 knots), following standard bongo techniques (Smith and Richardson 1977). Nets were towed obliquely from 200 m depth, or from within 5 m of the bottom,

TABLE 1. Summary of larval redfish (*Sebastes* spp.) data from Flemish Cap, 1978–82. Temperature is that of surface waters at 10 m depth at 47°N, 45°W.

Year	Date	Julian mid-date	Stations (*n*)	Larval abundance	Average larval length (SL) April (mm)	Average larval length (SL) June–July (mm)	Temp. (°C)
1978	16–23 July	201	56	2.7×10^{11}	18.5	9.2	11.5
1979	20–24 March	80	42	1.1×10^{11}	5.6		4.2
	23–27 April	115	42	6.9×10^{12}	6.3		5.2
	10–14 July	193	20	1.1×10^{11}[a]	—	6.3	12.6
1980	6–13 April	101	41	8.0×10^{11}	6.4		3.4
	20–26 May	145	42	2.2×10^{12}	8.9		6.1
	22–28 July	207	56	1.1×10^{11}	17.9	10.3	11.6
1981	2–9 May	126	41	6.1×10^{12}	8.3		6.3
	22–27 May	145	42	2.7×10^{12}	9.0		7.3
	26–30 June	179	42	3.6×10^{11}	11.3	6.5	10.3
	1–4 August	215	20	1.2×10^{10}[a]	21.4	15.2	12.7
1982	1–3 August	214	20	9.5×10^{10}[a]	20.2	10.7	11.5

[a]Estimate for a 42-station grid assuming that these 20 stations sampled 86% of the larval *Sebastes* spp. on Flemish Cap during July.

with payout and retrieval rates of 0.83 and 0.33 $m \cdot s^{-1}$, respectively. Each net was fitted with a General Oceanics flow meter. In 1978 and 1979, paired nets of 0.505- and 0.333-mm mesh sizes were used. In all subsequent surveys only 0.333-mm mesh nets were used. Maximum depth was monitored using a pressure sensor fitted above the bongo frame. During two surveys, 20–24 March and 10–14 July 1979, oblique tows were taken down to 125 m depth only.

Samples were preserved in 5% buffered formalin. All fish eggs and larvae were sorted from each paired sample and identified to species, or lowest taxonomic level possible. No attempt was made to distinguish the possible three species of redfish reported for Flemic Cap (Templeman 1976). Larval fish were counted and measured to the nearest 1 mm standard length.

Abundance was calculated as number of larvae per square metre:

$$N = CD/\Pi r^2 L$$

where C is the number of larvae collected, D is the maximum sampled depth (metres), L is the length of the two path (metres), and r is the radius of the net opening (metres). Total abundance for the study area was calculated as the summed abundance for a 42-station grid, where each station was taken to represent approximately 1.37×10^9 m^2. Where only 20 stations were sampled during July 1979 and August 1981 and 1982, total abundance for a 42-station area was estimated, assuming 86% of all larvae had been sampled by 20 stations over central Flemish Cap. This was based on a comparison with full areal coverage during 1978 and 1980 (Anderson 1981).

Larval growth was calculated as the increase in the average size of larval redfish sampled between one survey and the next, per unit time (Julian days). Only post-peak release period values are used, eliminating significant reductions of population growth through birth. Further, it is assumed that there are no significant larval migrations into the study area. Therefore, this calculation of growth contains elements of physiological growth and population mortality.

Oceanographic data were collected at each station, using a Guildline Mark IV CTD probe fitted to a Rosette sampler. Sampling was carried out to within 5 m of the bottom, down to maximum depths of 1000 m. Water temperatures sampled at 10 m depth over central Flemish Cap (47°N, 45°W) were taken to be representative of surface water conditions. This was based on a mixed-layer depth of ~20 m during all surveys and waters at this central location being most representative of the Flemish Cap surface waters (Keeley 1982).

Long-term temperatures in surface waters over Flemish Cap were calculated as mean monthly values from two data sources. One was estimated sea surface temperatures based on METOC charts for the position 47°N, 44°30′W (W. Bailey, Atlantic Oceanographic Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S., unpubl. data), while the second was based on the Marine Environmental Data Services' bathythermograph and Nansen bottle data at 0 and 10 m within the area bounded by 46°40′–47°20′N and 44°30′W–45°20′W (cf. Keeley 1982). The METOC data represented an observation for each mid-month from 1962 to 1981, while the MEDS data set represented all data available for 1910–81, by month. Values from both sources were combined to give mean monthly temperatures and standard deviation.

Results

Larval redfish constituted at least 90% of all larvae sampled during the 12 surveys. Abundances estimated for the survey area ranged through two orders of magnitude during different phases of the release cycle (Table 1; Fig. 2). Values during the early part of the cycle in late March 1979 and early April 1980 were 1.1×10^{11} and 8.0×10^{11} larvae for the survey area of 42 stations, respectively. Abundance increased quickly to peak values estimated by linear extrapolation to be $\sim 9.2 \times 10^{12}$ larvae occurring at the end of April, first week of May. Observations close to this estimated peak during late April 1979 and early May 1981 were 6.9×10^{12} and 6.1×10^{12} larvae, respectively. Abundances decreased exponentially from this peak period to lowest values of $< 3 \times 10^{11}$ larvae observed during July and early August.

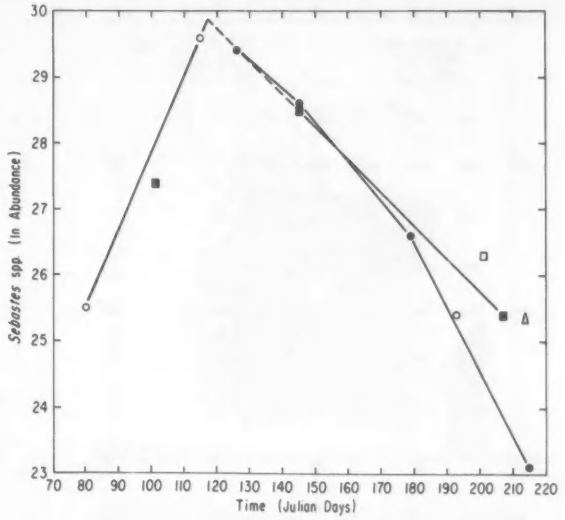

FIG. 2. Abundance estimates for larval redfish (*Sebastes* spp.) sampled on Flemish Cap, 1978–82. □, 1978; ○, 1979; ■, 1980; ●, 1981; △, 1982.

The abrupt decline in the numbers of 6 ± 1 mm redfish larvae indicates a pronounced period of maximum larval release. A similar short duration of peak redfish release has been reported in the Irminger Sea (Jones 1970). The result is a distinct unimodal peak containing the bulk of larvae released during any one year. It is not known how variable the time of maximum release is on a yearly basis, but it appears to occur about the last week in April on Flemish Cap.

The decreases in abundance observed during May–July corresponded to larval population mortalities that were in excess of 99%, based on the single release at the end of April. Average exponential mortalities calculated through this period ranged form 0.043 to 0.093·d^{-1} in 1980 and 1981 ($Z = (\ln N_0 - \ln N_1)/\Delta t$). In 1981, mortality rates increased from 0.04 between May surveys to 0.06 between late May and June to a high of 0.13 between late June and the first week of August. As well, 1981 mortalities were almost twice as high as mortalities during the same May–July period in 1980. Average mortality from 2–9 May to 1–4 August 1981 was 0.070.

Year-to-year comparisons of abundance are best made during July–August. A direct comparison can be made between August 1981 and 1982. Here, larvae >13 mm were an order of magnitude more abundant in 1982 than in 1981 (Table 2). During 16–28 July 1980 abundance was 8.4×10^{10} larvae. This compares closely with the value of 8.7×10^{10} estimated for 1982, a date only 7 d later. Using an estimated mortality coefficient of 0.05 for 1980, abundance 7 d later (i.e. the same mid-date as 1982) would have been 5.9×10^{10} larvae, slightly lower than 1982. A direct comparison with 1978 abundance is not possible; there was no mortality estimate for 1978 and the survey occurred 14 d earlier than 1982. However, given the high abundance estimated for 1978 and assuming even relatively high mortality coefficients, it appears that 1978 was the most abundant year for larval redfish. For instance, mortality coefficients from a number of studies have ranged from 0.03 to 0.08 (Anderson 1981). Using the highest coefficient, 1978 abundances on Julian day 214 would have been 9.2×10^{10} larvae for that date.

TABLE 2. July–August abundances estimated for larval redfish (*Sebastes* spp.) >13 mm on Flemish Cap.

Year	Sample mid-date	42-station abundance	42-station abundance for Julian day 214
1978	201	2.8×10^{11}	1.4×10^{11}[a]
1979	193	Nil	Nil
1980	207	8.4×10^{10}	5.9×10^{10}[a]
1981	215	7.9×10^{9}	7.9×10^{9}
1982	214	8.7×10^{10}	8.7×10^{10}

[a]Estimated by extrapolation using $N_1 = N_2 e^{-Zt}$, where $Z = 0.05$.

Based on these comparisons one might rank abundance of larvae released in April–May during 5 yr of surveys on Flemish Cap, in decreasing order of abundance, as 1978, 1982, 1980, 1981, and 1979. The years 1978, 1980, and 1982 were all an order of magnitude greater than in 1981, while 1979 larvae >11 mm were not observed on Flemish Cap in July.

The early distribution of redfish larvae was localized in the southwest corner of Flemish Cap in March 1979 over depths >300–400 m (Fig. 3a). Larval redfish at this time were highly unimodal around 6 mm (Fig. 4) and low in abundance (Table 1; Fig. 2). As the season progressed into April and the rate of larval release increased, maximum concentrations of recently released 6-mm larvae were observed around Flemish Cap in waters >200 m depth (Fig. 3b and 3c). There were very few larvae in the shallow waters overlying central Flemish Cap.

Release of larvae 2–9 May 1981 was past the date of peak extrusion as evidenced by the low numbers of 6-mm larvae (Fig. 4). At this time larval redfish were abundant and widely distributed throughout the survey area, with remnant low abundance still observed in the central area (Fig. 3d). Observations available through the rest of May, June, July, and into August demonstrated that larval redfish tended to occur in waters generally within the 400-m contour (Fig. 3e–3l). The presence of 6-mm larvae during June, July, and August indicates that larval release continues through this period. During three years, 1978, 1979, and 1981, summer distributions were highest in small areas north and west of the 200-m contour (Fig. 3g, 3h, 3i, and 3k). In July 1980, larval redfish concentrations were highest in the central area of Flemish Cap over waters <200 m depth (Fig. 3j). In August 1982 there was little pattern in the abundance distribution, although all three zero observations occurred at the margins of the area sampled (Fig. 3l).

To summarize, the major area of redfish larval release took place in waters >200 m depth, beginning first in the southwest corner and later mostly over the broad area lying immediately to the north and west of the central, shallow area. Following peak extrusion, occurring in late April, there was an apparent shift in the abundance distribution towards the central waters of Flemish Cap. This might be the result of convergence, or of higher survival over the central area.

Length–frequencies of larval fish sampled during March and April were unimodal around 6 mm SL (Fig. 4). Average size ranged from 5.6 to 6.5 mm (Table 1). Thus, recently extruded redfish larvae, formalin preserved, were approximately 6 mm in length. This was true of larvae released during June and July as well (Fig. 4). Even by 23–27 April 1979, it is apparent that mass extrusion of larve was still occurring, with little overall increase

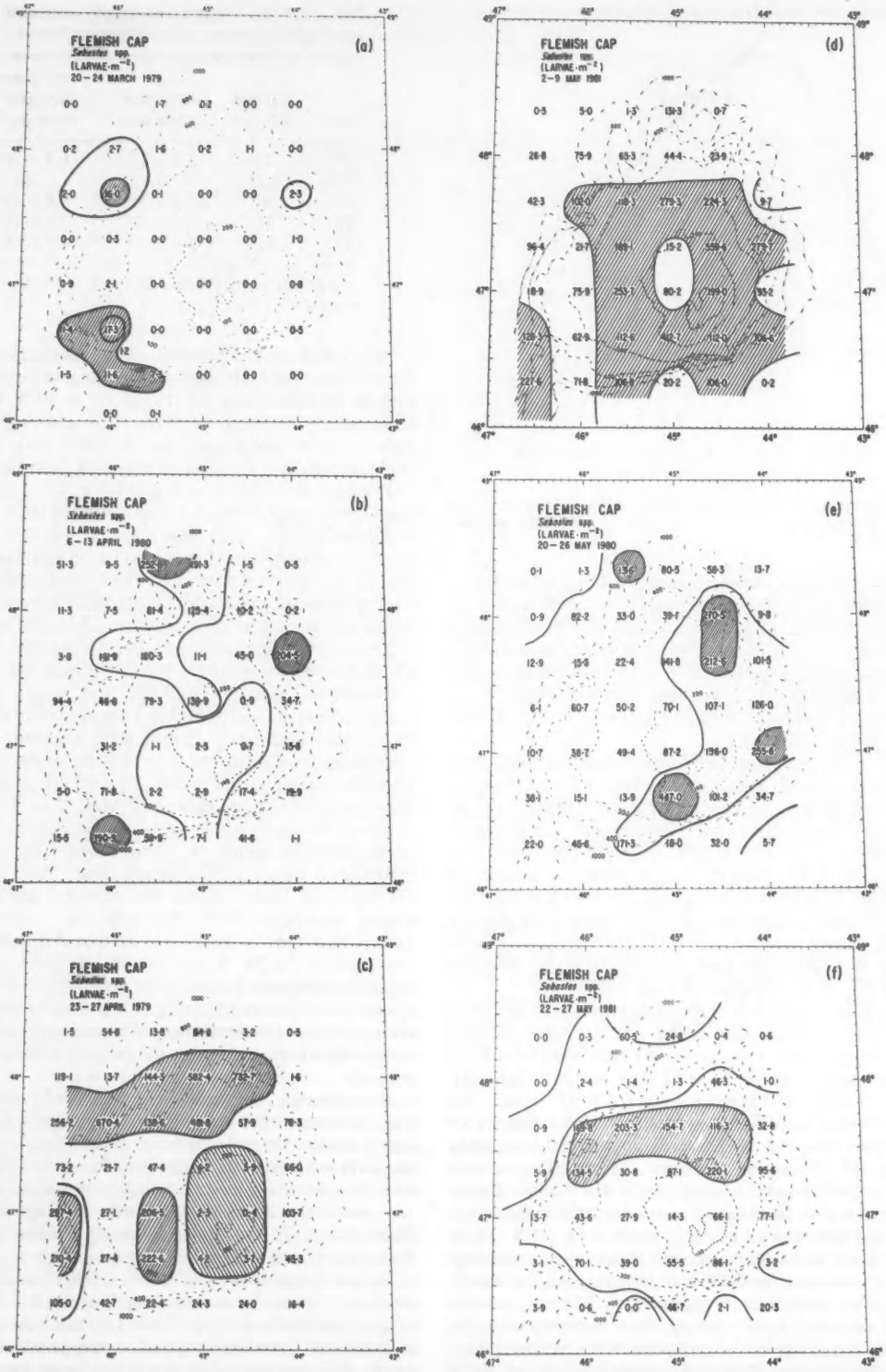

FIG. 3. Abundance distributions (larvae·m^{-2}) of larval redfish (*Sebastes* spp.) sampled on Flemish Cap, 1978–82.

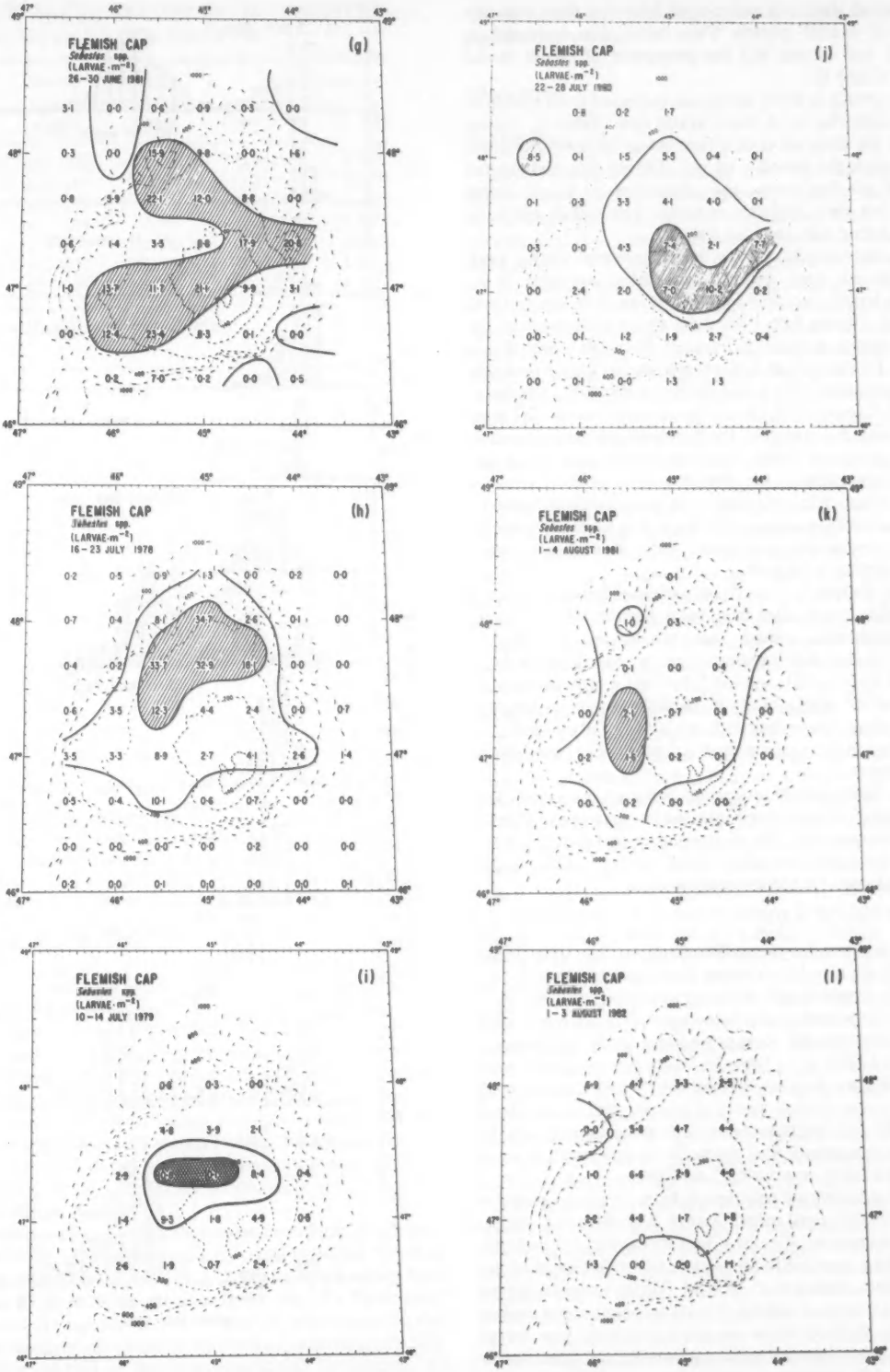

FIG. 3. (*Concluded*)

in mean larval size. It was not until May that there was any evidence of overall growth when mean size increased to between 8 and 10 mm and the proportion of 6-mm larvae decreased (Table 1).

During surveys in 1981, larval size increased from a mean of 9.0 mm in late May to 11.3 mm in late June (Table 1). During June 1981 the proportion of 6-mm larvae increased slightly, compared with the previous survey. During July and August surveys of all five years, the abundance of 6-mm larvae decreased but their presence indicates that redfish extrusion continues during July and into August.

In July and August, larval redfish released during peak extrusion in late April, possibly early May, averaged 18.0–21.4 mm in length, except in July 1979 when no larvae >11 mm were found. During July 1980 there was a third mode in the larval distribution at 10 mm SL, ranging from 8 to 12 mm (mean 10.3 mm). This mode may indicate a second extrusion peak that occurred sometime during late June or early July. Similarly, during 1–3 August 1982, 10- to 11-mm larvae (mean 10.7 mm) were observed. It is assumed that these peaks were produced as a result of a second redfish extrusion during June. However, evidence demonstrating the presence of a second extrusion period is limited. While the length–frequency data and proportion of 6-mm larvae suggest that there was a second peak in some years, numerical abundances during these periods were low and sampling infrequent.

Back-calculations to 6 mm SL of estimated larval abundance of the smaller length sizes from July–August data, based on average exponential growth (see below) and a mortality coefficient of $Z = 0.05$, indicated peaks around Julian dates 160–172 in 1978, 1980, and 1982. In 1981 a peak occurred at day 117 (end of April), while in 1979 there was no apparent peak. Therefore, these data indicate a peak, albeit small, in redfish extrusion during the second to third week of June in three of the five years.

Average larval length estimated for each sampling date indicated that growth of April-released larvae was exponential from May through July. Due to ongoing extrusion during April there was an initial lag after which average larval length increased. Growth fits the exponential form

$$(1) \quad L = 2.2 \exp(0.01D)$$

where L = standard length (millimetres) and D = time (Julian day) ($r^2 = 0.96$, $n = 11$). Average sizes for June–August were based on the largest length–frequency distributions (Fig. 4).

Increases in average length following April extrusion in 1980 and 1981 corresponded to exponential growth rates ranging from 0.40 to 1.66%·d^{-1} (Table 3). While average growth rates estimated for May–July in 1980 and 1981 were similar, growth rate in 1981 was slightly lower (Table 3). Values calculated from the 1981 data indicate that growth rate decreased initially followed by an increase. This appeared to be the result of lower than expected larval size during June 1981.

There was a significant relationship between mean larval size of April–May-released redfish larvae and observed surface water temperatures on Flemish Cap ($P < 0.005$, $r^2 = 0.86$); exclusion of the June 1981 observation, which fell substantially below the line, increased r^2 to 0.96. While compounded by other factors, seasonal heating of surface waters could explain most of the increase in larval size. Estimated size versus temperature from a second release period indicated no significant relationship; however, dropping the July 1979 observation resulted in a highly significant regression ($P < 0.01$, r^2 =

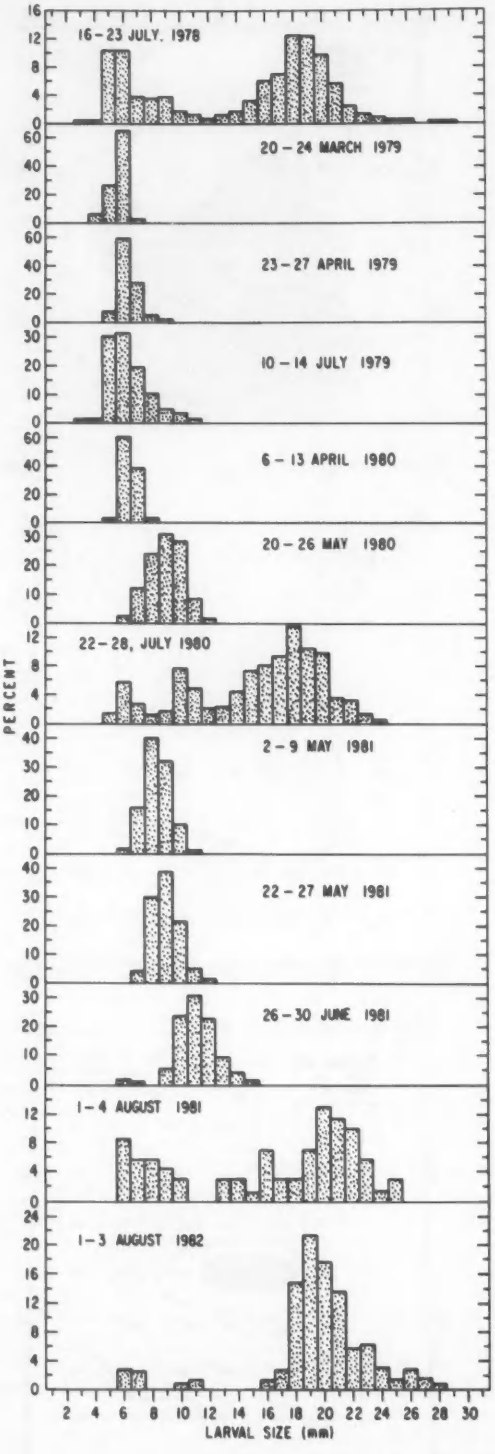

FIG. 4. Length–frequency distributions for larval redfish (*Sebastes* spp.) sampled on Flemish Cap, 1978–82.

TABLE 3. Size and growth for April–May-released larval redfish (*Sebastes* spp.) from Flemish Cap.

Year	Period (Julian dates)	Growth increment (mm)	Growth rate ($mm \cdot d^{-1}$)	Growth rate ($\% \cdot d^{-1}$)
1980	145–209	9.04	0.146[a]	1.13[a]
1981	126–145	0.7	0.037[a]	0.87[a]
	126–179	2.91	0.055	0.56
	145–179	2.30	0.068[a]	0.40[a]
	126–215	12.10	0.136	1.01
	145–215	11.40	0.163	1.05
	179–215	9.19	0.255[a]	1.66[a]

[a]Actual between-survey estimates.

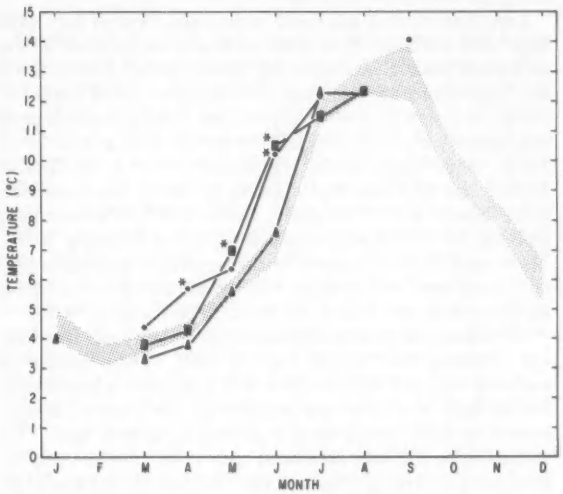

FIG. 5. The 99% confidence interval (shaded portion) of the long-term mean monthly temperatures of surface waters (≤10 m) for central Flemish Cap (46°40′–47°20′N by 44°30′–45°20′W). Plotted also are mean monthly temperatures (≤10 m depth) for 1979–81. Asterisks indicate significant differences from the long-term mean ($P < 0.01$). ●, 1979; ▲, 1980; ■, 1981.

0.95). Thus, while the general fit of larval size versus surface water temperatures was good during these three years, there were two cases where it did not hold: June 1981 and July 1979. In both cases, lower than expected mean population sizes were associated with higher than average surface water temperatures.

The long-term averaged temperature of surface waters on Flemish Cap increased nonlinearly from May through July (Fig. 5); temperatures increased gradually from a minimum of 3.3 ± 0.3°C in February to 5.0 ± 0.4°C in May, and rose rapidly to 11.5 ± 0.3°C in July. Maximum temperatures of 12.7 ± 0.6°C occurred during August.

Comparison of mean monthly temperatures form all available data for 1979–81 with the long-term mean indicated only four significant differences ($P < 0.01$). These occurred during April and June 1979 and May and June 1981 (Fig. 5). While other values were not significant at the 99% level, it is noteworthy that all mean monthly values during 1979 were greater than the 99% confidence interval of the long-term mean. In contrast, no values from 1980 were significantly different from the long-term mean. Thus, 1979 was a warmer than average year throughout the spring heating cycle, with April and June being significantly warmer.

Discussion

Redfish Populations

Larval redfish data collected over 5 yr on Flemish Cap demonstrated there are probably two periods of larval release: one during April–May and a second during June–July. These periods of release correspond with two cycles in maturation of adult redfish on Flemish Cap (Templeman 1976). Whether or not these release periods represent separate populations remains a matter of conjecture. Insemination in redfish takes place many months earlier, probably during the fall (cf. Westrheim 1975; Jones 1970). However, virtually nothing is known about this aspect of redfish life history, e.g. whether there are different mating times, different locations, or different depth zones. In addition, on Flemish Cap three species of redfish are reported (Templeman 1976). Of these, *Sebastes mentella* is the most numerous and releases its larvae during April–May (Templeman 1976). Extrusion of *S. fasciatus* occurs later in May or sometime in June (Barsukov and Zakharov 1972; Templeman 1976). The extent to which this forms a second peak will depend on abundance of *S. fasciatus* compared with *S. mentella*, as redfish extrusion appears to be continuous from April to possibly September or October in many areas (Barsukov and Zakharov 1972). The lack of knowledge regarding the mating of redfish species and the confused nature of redfish species identification, coupled with possible interbreeding between species (Templeman 1976), makes it difficult to discuss population differences between these redfish.

A bimodal spawning peak for Flemish Cap is similar to that reported in the Irminger Sea, a large oceanic area lying southeast of Iceland, and in contrast with that reported for all areas lying west of Flemish Cap. In the Irminger Sea, redfish first release larvae in April–May, followed by a second, smaller release during June and July (Magnùsson 1968). These extrusions are thought to be species specific, attributed to *S. mentella* and *S. viviparous*, respectively. Nowhere else in the North Atlantic have bimodal release periods been observed. On the adjacent Grand Banks and off Labrador, release first occurs during June (Bainbridge and Cooper 1971). In the Gulf of St. Lawrence release begins in May, with a peak occurring during early June (Steele 1957). On the Scotian Shelf, release occurs from April through July with a peak in June (Day 1961; Serebryakov 1962; Henderson 1968; Bainbridge and Cooper 1971), while in the Gulf of Maine, release occurs from May through July, with a single peak during late June (Kelly and Barker 1961). Thus, throughout the entire area lying to the west of Flemish Cap, from the Gulf of Maine north to the Labrador Shelf, the main release period of redfish larvae is unimodal and occurs significantly later than on Flemish Cap.

This observation supports the idea that Flemish Cap constitutes a separate stock complex (Mead and Sinderman 1961; Bainbridge and Cooper 1971; Templeman 1976; Ni 1982). The pattern of release indicates that the redfish assemblage on Flemish Cap is distinct from the adjacent Grand Banks, and more closely associated with that of the Irminger Sea, as pointed out by Bainbridge and Cooper (1971). Oceanographically, Flemish Cap is a distinct area, bounded by the Labrador Current to the north and west and the North Atlantic Current on the south and east. Cod on Flemish Cap also constitute a separate stock.

Discontinuous larval concentrations over Flemish Cap support the notion of a discrete larval population. As such, the early life history of these fishes will be dependent on the vicissitudes of the Flemish Cap ecosystem.

A second question of interest concerns different release strategies for redfish distributed at similar latitudes. On the Cap, release is bimodal with most of the annual redfish release occurring in April at the onset of seasonal production cycles. Elsewhere, the spawning strategy is unimodal with the main release of larvae occurring about 2 mo later, well after spring production peaks. In all cases, two or more species reportedly occur in each area. The release periods may therefore be species specific. On both Flemish Cap and in the Irminger Sea the dominant redfish is reported to be *S. mentella* and the main release period occurs during April and early May. In the other areas, *S. fasciatus* dominates and is reported over the shelf areas and upper slope, while *S. mentella* occurs in deeper waters (Ni 1982). On both the Grand Banks (Mobil Oil Canada Ltd., P.O. Box 62, St. John's, Nfld., unpubl. data) and the Scotian Shelf (R. O'Boyle, Marine Fish Division, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.) redfish release begins in April along the shelf break areas, with redfish larvae appearing in shelf waters only later on in May and June. The conclusion is that the redfish larvae released early are *S. mentella*, and those occurring during the June peak are *S. fasciatus*.

Larval Growth and Mortality

Mean larval size of April–May-released larvae increased exponentially throughout the periods sampled, following an initial lag phase due to ongoing extrusion. With the exception of June 1981, all observations fit this curve well, which is the expected case for early growth of fish larvae (Ahlstrom 1954; Nakai and Hattori 1962; Houde 1977). At some point, however, growth rate will drop off coincidental with metamorphosis to a juvenile stage. In the Gulf of Maine, for June-released redfish larvae, this was reported to occur at 40–50 mm length during October–November (Kelly and Barker 1961). This occurs from 30 to 55 mm in several species of Pacific redfish, but mostly around 50 mm (Moser and Ahlstrom 1978; Laroche and Richardson 1980; Boehlert 1981; Boehlert and Yoklavich 1983). If this was true for Flemish Cap, similar lengths would be reached about the third week of October. With this assumption, average growth rate for larval redfish would be of the order $1.11\% \cdot d^{-1}$ from May through October.

These growth rates are similar to those of other studies. Unpublished data collected on Flemish Cap during May, June, and July 1978 (Postolaky 1980) indicate that growth ranged from 1.25 to $1.32\% \cdot d^{-1}$ from May through July. Values for the May–July period ($1.29\% \cdot d^{-1}$) were slightly higher than 1980 and 1981 estimates for approximately the same period. From this study, the growth rate calculated for June 1981 was $0.40\% \cdot d^{-1}$, considerably less than the rate of $1.32\% \cdot d^{-1}$ estimated from Postolaky's data. However, values during July 1981 were higher, being $1.66\% \cdot d^{-1}$ compared with $1.25\% \cdot d^{-1}$ for the 1978 data. Thus, the low growth rates observed during the early part of the growing season in 1981 did not occur in 1978. This was primarily due to the comparatively larger size of redfish larvae sampled during June 1978 (14.5 mm) as compared with May (9.3 mm) and July (20.1 mm) of that year.

Growth rate data for larval redfish can also be estimated from the Labrador Shelf and the Gulf of Maine. During July–August on the Labrador Shelf, larvae grew from about 8 to 14.5 mm in 30 d (Bainbridge and Cooper 1971) corresponding to a rate of $\sim 1.98\% \cdot d^{-1}$. In the Gulf of Maine, redfish larvae grew at a rate of $1.22\% \cdot d^{-1}$ during July–August to September, increasing from 23.6 to 38.5 mm in 42 d (Kelly and Barker 1961). These estimates are marginally greater than those for Flemish Cap and certainly greater than estimates for May–June 1981. Possible differences may be species related and/or due to different environmental conditions experienced by the later release periods in these other areas. None of these estimates approach the low growth rates from 1981. This was also true compared with growth estimates for the Pacific redfish, *Sebastes diploproa*, which ranged from 0.154 to $0.225\ mm \cdot d^{-1}$ based on otoliths of larval fish ranging from 9.0 to 42.7 mm SL and a variety of surface temperatures, which varied from 13 to 22°C (Boehlert 1981) (cf. Table 3).

Larval mortalities estimated from April through July were high, with less than 1% of larvae estimated for the study area at the time of peak release surviving. Such rapid declines in larval fish populations are common. For example, larval studies of North Sea plaice (*Pleuronectes platessa*) over a number of years indicated 94.8–99.6% mortality over 140 d (Bannister et al. 1974), while round herring (*Etrumeus teres*) in the Gulf of Mexico had 98.3 and 99.4% mortality during two successive years (Houde 1977). Similarly, mortalities >99% have been reported for cod, plaice, and herring larvae (Cushing 1974). These rapid declines in abundances are usually associated with decreasing rates of mortality, with proportionally more larvae surviving with time (Bannister et al. 1974; Cushing 1974).

An unusual increase in instantaneous mortality rate with time was observed on Flemish Cap in 1981. These increasing mortality rates in 1981 coincided with a decreasing growth rate during May and June and suggest an association between increasing larval mortality and decreasing growth rates. This appears to be the case compared with 1980. Similar observations for growth over May and June in 1980 are not available. However, abundances that were almost identical during the last week of May 1980 and 1981 differed by an order of magnitude in July (Table 1). Additionally, growth rates estimated during May and June 1978, a year of high larval abundance (Table 2), indicated higher growth compared with 1981. It appears, then, that low growth rates during May and June of 1981 may have been significantly low and that these low and decreasing growth rates led to high larval mortality.

It appears that extremely high larval mortality observed in 1979 also occurred during May–June. Following what appeared to be a normal release period in April, with abundances close to the predicted maximum on Flemish Cap (Fig. 2), there was a total absence of these larvae (>11 mm) in July. Between our April and July sampling dates in 1979 there appears to have been 100% mortality of larvae from the first release period. In addition, length–frequencies during July indicated no increase in mean size, as compared with other years. This high mortality is somewhat confirmed by cod feeding studies routinely carried out during January of each year. Samples collected in January 1980 indicated an almost complete absence of 7- to 8-cm redfish in cod stomachs, assumed to be O-group fish (Lilly and Gavaris 1982). This was in contrast with feeding studies in other years, both before and after 1980, in which young redfish 7–8 cm in length comprised a significant portion of the cod diet.

From these data, then, there is evidence of high larval mortality that occurred sometime during May–June 1979 and again in 1981. In addition, this may have been growth related,

higher mortality occurring when growth was reduced. Finally, the magnitude of this response varied between years. This suggests that if there is a critical period for larval redfish survival on Flemish Cap, it occurs sometime during May–June.

Recruitment Variability

Determining causes of recruitment variability was of underlying importance to this study. Over the course of 5 yr, July abundances of redfish larvae varied widely. The most significant observation was the total mortality of the April–May-released redfish in 1979. While surface water temperatures over central Flemish Cap correlated highly with larval size in most cases, it did not during June 1981 or July 1979. These observations indicate that larval growth and survival may have been affected by seasonal heating that was excessive during June.

Physiologically there is a sound basis to expect that larval growth would be under the direct control of water temperatures, within tolerance bounds and where food was not limiting (Brett et al. 1969; Brett and Groves 1979; McCormick et al. 1971; Ryland et al. 1975; Ryland and Nichols 1967). In this case one might expect that larval growth on Flemish Cap would respond directly to the seasonal heating cycle. Obviously such a relationship is compounded by other factors, such as increasing daylight and hence time available to feed, successional community changes affecting food supply, and increased water stability, which may act to concentrate food. However, such a relationship with temperature will break down outside certain limits. In the case of upper temperature tolerances, metabolic demands increase, initially resulting in reduced growth and ultimately in death (Brett et al. 1969; Ryland and Nichols 1967; Ryland et al. 1975; Williams and Caldwell 1978; Boehlert 1981). Compounded in these considerations are rates of change, which we know little about. An abrupt change in temperature would have detrimental effects on growth and survival in fish larvae (Colton 1959; Brett and Groves 1979).

On Flemish Cap not only were temperatures during June 1979 and 1981 significantly warmer than the long-term mean but these also occurred during the period of greatest heating of surface water. An estimated 48% of the annual increase in surface water temperatures occurs from June to July. It is possible that the magnitude and rate of seasonal heating of surface waters on Flemish Cap may have been responsible for high larval mortalities during these years. It may be that this particular period, through which there is a physiological hurdle imposed by exponentially increasing temperatures, may have constituted a critical period for larval fish survival.

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NOTES

Mortality Rate of Fishes in the Pelagic Ecosystem

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Peterson, I., and J. S. Wroblewski. 1984. Mortality rate of fishes in the pelagic ecosystem. Can. J. Fish. Aquat. Sci. 41: 1117–1120.

A size-dependent equation for mortality rate ($M(w)$) of fish-sized particles is derived using preexisting theory on the distribution of biomass as a function of size (w) in the pelagic marine ecosystem, assuming that mortality is primarily due to predation. The equation $M(w) = ckw^{-x}$ (where c, k, and x are parameters) yields estimates that are close to observed mortality rates.

On dérive une équation dépendante de la taille pour le taux de mortalité ($M(w)$) des poissons, à l'aide d'une théorie élaborée antérieurement et selon laquelle la répartition de la biomasse est fonction de la taille (w) dans un écosystème marin pélagique, en tenant pour acquis que la mortalité est principalement due à la prédation. L'équation $M(w) = ckw^{-x}$ (où c, k et x sont des paramètres) génère des estimations qui s'approchent des taux observés de mortalité.

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Estimation of natural mortality rate for fish populations is critical for the prediction of recruitment and for the optimal management of the recruited stock. As pointed out by Cushing (1974), numbers are reduced by four to seven orders of magnitude between hatching and recruitment. Mortality rate of fish larvae and juveniles is known to decrease with increasing size (Pearcy 1962; Cushing 1974), although the underlying basis for this relationship is not clear. In addition, adult mortality rates for different species decrease with maximum size (Ursin 1967). A general theory for mortality of fishes based on broad marine ecosystem processes may be helpful in understanding these trends.

Organism size has long been used as a scaling factor for such biological processes as growth and metabolism (Fenchel 1974). This has been applied in the development of a theory to explain the distribution of biomass among size-classes in the pelagic marine ecosystem (Kerr 1974; Platt and Denman 1977, 1978; Silvert and Platt 1978, 1980). In one analysis, Silvert and Platt (1980) gave an expression for mortality rate due to predation, but did not evaluate it explicitly.

Their expression (equation (21)) has been used here to derive a size-dependent equation describing mortality rate of fish-sized particles, and the equation is compared with data collected by other workers.

General Equation

The size distribution of organisms in the pelagic food chain can be described by the following time-dependent equation (Silvert and Platt 1980):

$$(1)\quad \frac{\partial \beta}{\partial t} + w\frac{\partial}{\partial w}(\beta G) + \beta M = 0$$

where $\beta(w, t)$ is a biomass density function such that $b(w, t) = \beta(w, t)dw$ is the mass of particles per unit volume in the size interval from w to $w + dw$ at time t. Silvert and Platt (1980) assumed predation scales in a perfectly isometric fashion, i.e. the range of particle sizes acceptable as prey scales as w. Thus, $dw \sim w$.

The function $G(w)$ is the specific growth rate of particles, i.e. $\partial w/\partial t = G(w)\cdot w$. Lastly, the function $M(w)$ is the specific rate of change in numbers of particles due to mortality and reproduction, i.e. $\partial N/\partial t = -M(w)\cdot N$ where $N = \beta/w$.

Silvert and Platt (1980) solved equation (1) after making the following assumptions: (1) the specific rate of change in numbers, $M(w)$, is primarily due to predation, (2) the weight of prey is a constant fraction q of the weight of the predator, and (3) the system is at steady state, i.e. $\partial\beta/\partial t = 0$.

According to the first assumption, production of particles within the spectrum through reproduction must be insignificant. To meet this condition, we will consider the biomass spectrum only above the size corresponding to the size of fish eggs, which is relatively constant between species (Ware 1975). There is a constant influx of material at the lower end of the spectrum.

Silvert and Platt (1980) showed that one possible solution to equation (1) is

$$(2)\quad \beta(w)G(w) \sim w^{-c}$$

where the exponent c is such that

$$(3)\quad K_1 c = q^{c-1}$$

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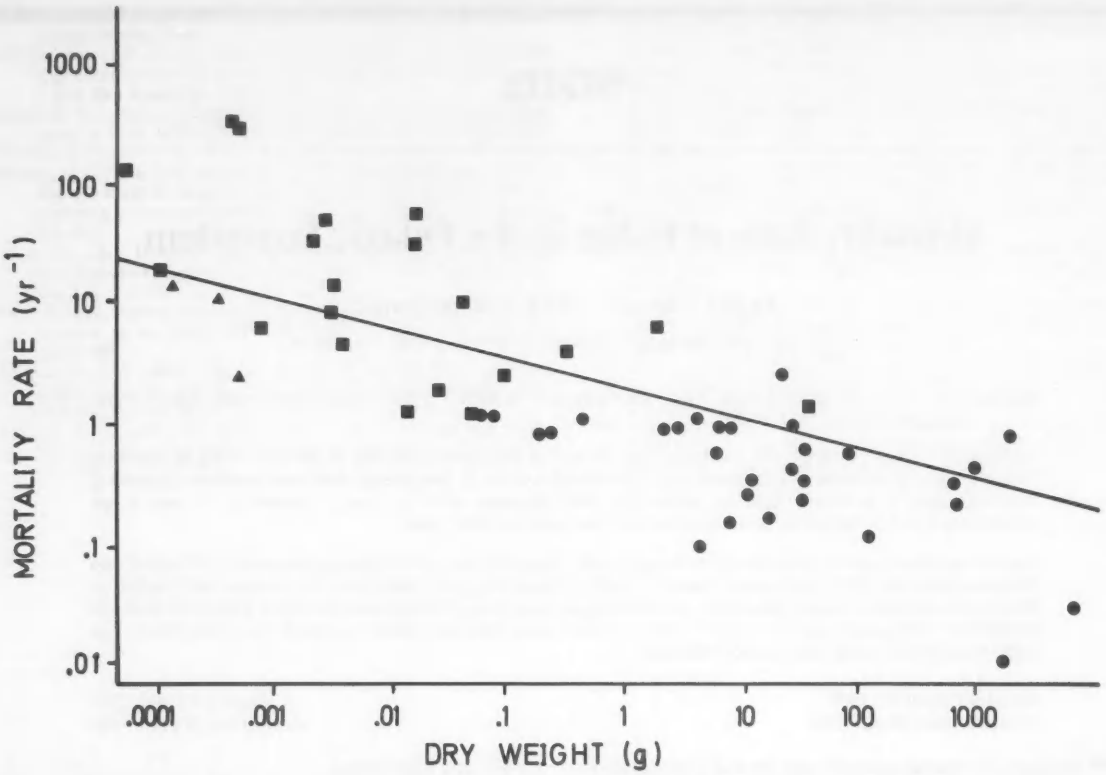

FIG. 1. Natural mortality rate vs. dry weight. The straight line is described by equation (8). ●, adult fish (Ursin 1967); ■, larval and juvenile fish (Ahlstrom 1954; Cushing 1974; Farris 1961; Graham and Chenoweth 1971, 1973; Pearcy 1962; Poulsen 1931; Sette 1943); ▲, chaetognaths (Sameoto 1971).

and parameter K_1 is the growth efficiency, i.e. growth divided by ingestion. Specific growth rate can be expressed as

(4) $G(w) = kw^{-x}$

where k and x are constants (Kerr 1974), so that from equation (2),

(5) $\beta(w) \sim w^{x-c}$.

Using equations (20) and (21) from Silvert and Platt (1980),

$$M(w) = \frac{G(w/q)}{K_1 q} \frac{\beta(w/q)}{\beta(w)}$$

and from equations (4) and (5) above,

(6) $M(w) = \frac{k}{K_1} q^{c-1} w^{-x} = ckw^{-x}$.

To estimate parameter c in equation (3), we need to know the growth efficiency, K_1. Somatic growth represents the difference between assimilated ration and losses due to metabolism and reproduction. Metabolism is found to take the form αw^{γ} where $\gamma \simeq 1 - x$ (Kerr 1974). As will be shown below, reproductive losses are small relative to metabolic losses and therefore can be ignored. Thus, if I is the ingestion rate and p is the assimilation efficiency,

$$\frac{dw}{dt} = pI - \alpha w^{1-x} = G(w) \cdot w = kw^{1-x} \quad \text{for all } w.$$

Clearly, then,

$$I = \frac{k + \alpha}{p} w^{1-x}$$

and growth efficiency,

(7) $K_1 = \frac{p}{\alpha/k + 1}$.

Calculation of Biomass and Mortality Rate Parameter Values

The following values are used to solve for the growth efficiency, K_1. The weight coefficient for metabolic rate α is taken to be $2 \times 10^{-7} \cdot s^{-1}$ (Fenchel 1974). The weight coefficient for growth rate k is set at $5 \times 10^{-8} \cdot s^{-1}$, which is the geometric mean of values given in Ware (1980). Finally, Kerr (1974) gave a value of 0.7 for the assimilation efficiency p. Substituting these values of α, k, and p into equation (7), we obtain a growth efficiency K_1 of 0.14.

To solve for c, the prey predator size ratio q is taken to be 3.45×10^{-4}, assuming that prey are 7% of the predator's length (Ware 1978) and that weight is proportional to the cube of the length. There is some indication that q decreases with increasing size (Silvert and Platt 1980), but this will be ignored. Therefore, from equation (3), $c \simeq 1.22$.

Finally, to solve for natural mortality rate, we need a value for the weight exponent x for growth and metabolic rate. According to D. M. Ware (Pacific Biological Station, Nanaimo, B.C.,

TABLE 1. Results of the sensitivity analysis as percentage change in the mortality rate for perturbation in individual parameters.

Parameter	Perturbation −25%	−5%	+5%	+25%
α	−1.6	0.0	0.8	1.6
k	−23.2	−4.2	5.0	23.0
q	−0.8	0.0	0.0	0.8
p	2.5	0.8	0.0	−1.6

pers. comm.), x varies between 0.1 and 0.4, so that an average value of 0.25 is used here. A value of 0.25 agrees with Fenchel's (1974) value for the weight exponent of metabolism. Thus, equation (6) becomes

$$(8)\quad M(w) = (6.1 \times 10^{-8} \cdot s^{-1}) w^{-0.25} \quad \text{or} \; (1.92 \cdot yr^{-1}) w^{-0.25}.$$

Note that from equation (5), $\beta(w) \sim w^{x-c} \sim w^{-0.97}$ as shown by Silvert and Platt (1980). Since $b(w) = \beta(w)dw$ and $dw \sim w$, then $b(w) \sim w^{0.03}$. As the exponent of w is very small, the biomass is virtually the same in all size-classes, in accordance with observations by Sheldon et al. (1972, 1973).

It was stated earlier that reproductive losses are small relative to metabolic losses and can therefore be ignored. This can be shown in the following way. Using values of α, k, and x given above, an animal of 125 g would have respired 198 g over the preceding year, while an animal of 1000 g would have respired 1010 g. Since egg production averages about 16% of the weight of the animal for various fish stocks (Gunderson 1980), metabolic rate is 6–10 times higher than reproductive losses for animals between 125 and 1000 g.

A sensitivity analysis was performed to assess the effects of perturbations of the parameter values for α, k, q, and p on the mortality rate (Table 1). Clearly, mortality rate is relatively insensitive to all parameter values except that of the growth rate, k.

Comparison with Data

The relationship between mortality rate and dry weight given by equation (8) is compared with data from various sources (Fig. 1). Body wet weight was calculated from Ursin's (1967) formula giving the mean size $\hat{w}$ of fish from which mortality estimates were made. A dry weight to wet weight ratio of 0.2 was assumed. Although the preceding analysis refers to pelagic ecosystems, no attempt was made to identify and exclude data from demersal fishes. Data for chaetognaths are also included, since chaetognaths are of the size being considered here.

On the whole, the relationship given by equation (8) agrees well with the observations except for very small and very large w. For fish larvae, field estimates of mortality rate may be too large in some cases because of patch diffusion and increasing net avoidance with age. For very large w, mortality rate of the adult fish is lower than that predicted by equation (8) because of the lack of predation above a certain size.

Discussion

The theory described in Silvert and Platt (1980) and applied here to fish-sized particles involves several assumptions. First, it is assumed that mortality is primarily due to predation. This is probably reasonable in the case of small organisms, for although disease and senility are present, the proximal cause of mortality is usually predation (Cushing 1974). However, in the case of the largest organisms, mortality is obviously controlled by other factors. Second, it is assumed that there is a constant influx of material at the lower end of the spectrum, when in fact the influx varies seasonally. Finally, no provision is made for food energy gained from the benthic ecosystem.

The relationship between mortality rate and size (equation (8)) can only be viewed as a central tendency for organisms in an ecosystem as a whole and may not be applicable to individual species. In any size-class, certain species will be particularly vulnerable to predation and will therefore have a higher mortality rate. In addition, mortality rate for individual species appears to increase after sexual maturity is reached (Cushing 1975).

It appears from Fig. 1 that the magnitude of larval fish mortality is consistent with the hypothesis that predation is the primary source of mortality. This is of interest in light of recent attention given to food availability as a major factor affecting larval survival, particularly at the end of the yolk sac stage (see May 1974). Presumably, though, the condition of individual larvae affects their vulnerability to predation.

In critique, the general expression for fish mortality as a function of size, $M(w) = ckw^{-x}$, yields values that are encouragingly close to observed mortalities. Parameters k and x are well founded in bioenergetics, while c can be calculated. It is easier to measure these parameters in the laboratory than to estimate the mortality rate of fishes directly in the field. However, this expression does not offer a practical alternative to the traditional methods of mortality estimation. Its application is limited by the assumptions and approximations made in formalizing the theory from which it is derived. Nevertheless, the expression does serve to relate many observations simply and to increase our understanding.

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PERSPECTIVES

A Commentary on Environmental Impact Assessment for Large Projects Affecting Lakes and Streams[1]

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In this essay I look at environmental impact assessment as it concerns major projects influencing aquatic environments in Canada. Environmental impact assessment is a process aimed at guarding the public interest in the proper use of resources in the aggregate. Compromises are usually worked out on a local basis for each project. More widely conceived trade-offs are favored by all but practised by few. All projects go through various stages, all begin as vague schemes; some crystallize as firm proposals and then go through the assessment process to construction and a subsequent period of operation. Environmental impact assessment as a process should reflect this pattern of activity rather than being just the preparation of statements about projects that are imminent. I have given particular emphasis to the need for follow up on whether what occurred was what was predicted. Also I have stressed the importance of anticipating that some impacts will not be foreseen and, therefore, the necessity for making appropriate financial provision. Environmental impact assessment, as presently practised, does not make the contribution it might to environmental science. Impact assessment should not be seen as a substitute for research that would lead to new understandings of ecological systems and to new appreciations of what to look for in making environmental impact assessments.

La présente étude porte sur l'évaluation des effets environnementaux dans le cadre de grands projets ayant une incidence sur les milieux aquatiques au Canada. L'évaluation des effets environnementaux vise la protection de l'intérêt du public pour ce qui est de l'utilisation rationnelle des ressources. On arrive généralement à des compromis sur une base locale pour chaque projet. Tous sont en faveur des concessions mais peu en font. Tous les projets passent par diverses étapes en commençant par de vagues plans. Certains se cristallisent comme propositions fermes, puis passent par l'évaluation, la construction et la période d'application. En tant que processus, l'évaluation des effets environnementaux devrait réfléter ce schème d'activité au lieu de n'être que la préparation d'énoncés sur les projets imminents. L'auteur mais l'accent sur le besoin d'un suivi afin de déterminer si les faits correspondent aux prévision. Selon lui, il importe d'anticiper que certains effets ne seront pas prévus et donc qu'il est nécessaire de prendre des dispositions financières appropriées. Dans l'état actuel des choses, l'évaluation des effets environnementaux n'apporte pas à la science de l'environnement la contribution qu'elle pourrait y fournir. Cette évaluation ne devrait pas être perçue comme un substitut à la recherche menant à de nouvelles découvertes sur les systèmes écologiques et à la compréhension des facteurs à étudier lors d'évaluations des effets environnementaux.

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Environmental impact assessment in Canada has come a long way since the early 1950s, when many major developments proceeded with relatively little study of environmental effects. At that time, the proponents did not prepare formal impact assessments that had to be defended publicly, nor did they undertake to do much more than was legally required of them in minimizing impacts. Nowadays, any major development project is sure to be accompanied by substantial attention to environmental effects, often well in advance of the earliest construction and almost invariably at significant cost.

The changes that have taken place are a source of considerable satisfaction to those who many years ago were advocates of "multiple resource use" — a vague notion about maximizing the total benefits from an area by using it for several activities at the same time (fish *and* power, forests *and* wildlife). The contemporary policies and procedures for environmental impact assessment seem to provide a satisfactory routine for making better resource decisions. But because the process is becoming increasingly a matter of routine, it is perhaps timely to reflect on whether it really *is* leading to better decisions, whether it is efficient, whether it is leading to even better decisions in the future. It is my intent in this essay to remark on such matters from the perspective of ecology as a science, with the emphasis on large-scale projects. To do this I will use examples from

[1]Based on an address presented to the Pacific Congress, Dunedin, New Zealand, February 1982.

western Canada, ones with which I have had experience over the past 35 yr.

Project Orientation of Environmental Impact Assessment

Taking a broad view, environmental impact assessment is a process aimed at guarding the public interest in the proper use of resources in the aggregate. For example, if a project might exploit one resource at the expense of another, say a hydroelectric dam at the expense of a salmon population, then there is an obligation to find the best compromise, perhaps by sacrificing some kilowatts for some salmon or vice versa. Most resource conflicts take this form, with a consideration of the local options for a particular project.

It is often argued that the trade-offs should be more broadly conceived and that ideally the environmental impact process should be regionally oriented rather than to specific projects or proposals (Munn 1979). Thus, the salmon of one river might be completely sacrificed in the interest of obtaining cheap electric power, while the power potential of another river should be foregone "forever" in the interests of salmon. More comprehensively, over the long term it may be best to examine other ways of producing power and, to be logical about it, other ways of producing salmon.

In my experience, while these more generalized approaches are popular in classroom seminars, they usually fall on deaf ears. Power companies tend to have fixations on particular ways of producing power, and particular sites in mind for their next projects. From their points of view, the options for power production have already been explored, and they will carry on with their fixations until they are sufficiently frustrated that they are obliged to find other fixations. Salmon enthusiasts have a similar rigidity. Any river, anywhere, is important to them; to trade one away for a promise that another will be inviolate is to lose another battle in a war of attrition. Even if most salmon enthusiasts can be persuaded otherwise, the local ones, those most affected, will seldom relent. If there is to be compensation rather than mitigation, say in the form of a salmon hatchery, the locals want the hatchery on their river, not somewhere else. Thus, although it has many times been suggested that some rivers in British Columbia be dedicated to power production and others to salmon, no one to my knowledge has ever considered such a scheme as politically viable.

A similar sort of theme runs through a great majority of situations, whether they concern hydro power projects, irrigation schemes, land reclamation ventures, or more generally, water quality management or land use planning as a whole. If there is to be compromise, it is likely to be of the local variety — addressed to what can be done to mitigate effects on the watershed affected by the proposed project. The ostensibly more sensible route of more widely conceived resource trade-offs usually seems too much for our institutions to handle. Everybody is in favor of it, but nobody does it. Perhaps the underlying reason is that, while all may agree with the common sense of sacrificing one thing for another, none wishes to be the object of sacrifice, especially when those that benefit live somewhere else.

Nevertheless, there is a higher level of environmental assessment that is epitomized by studies in developing countries that evaluate the possible impact of national programs or policies (e.g. see Interim Committee for Coordination of Investigations of the Mekong Basin 1982). The developed countries have similar needs. In formulating a national energy policy for Canada, for example, environmental implications played a part in determining the mix of energy sources that was seen as desirable. Similarly, a national program to curtail the deleterious effects of acid rain might involve strategic considerations about the nature and the development of industry as well as regulations aimed at reducing the current level of discharge from acid-generating sources.

What distinguishes these more general levels of assessment from those at the local level is their programmatic perspective and their potential for major influence on the national standard of environmental quality. They may establish a jurisdictional procedural context in which local problems are addressed. (Indeed, they were instrumental in the genesis of the various pieces of environmental impact legislation now in place nationally and in the provinces.) In the words of Thompson (1980), "it is important to perceive governmental responsibilities in a context of management and stewardship of publicly-owned natural resources as well as one of regulation and enforcement of environmental protection measures."

In the day-to-day local consideration of environmental problems, these higher levels of program formulation and policy making are often alluded to, but the common pattern is for a project orientation and compromise on a local scale. The process of compromise begins with some sort of project development proposal that elicits an environmental impact assessment and proceeds through public hearings that lead eventually to a decision about whether the project should proceed and, if so, with what imposed constraints. The essential grist for this decision-making mill is the scientific, social, and economic information adduced in support of the environmental impact assessment, together with information supplied by government agencies or simply volunteered by interested parties at public hearings. The public has abundant opportunity to participate.

Questions related to the intrinsic economics of the enterprise are usually left to the project developer. If, for example, a project is proposed for which there is no environmental impact of concern, the developer is usually left free to gamble his money on what may prove to be an uneconomic enterprise. The developer's economics only become a factor if it is claimed that the imposition of too many environmentally related expenses may make the project uneconomical. In this case, the adjudicators may profess a need to examine the economic arguments on the development side of the equation, but the usual approach is to ask how much environmental cost can be borne rather than whether there really is a market for the product or whether there are cheaper alternatives for doing the project. When government proposes a project, it seldom gets off so easy. The public usually wants to know whether the project is needed, as well as what the environmental impact may be, an appropriate attitude when it is their money.

The environmental impact assessment is evidently a crucial document in the decision to proceed, modify, or defer a project. For details of the procedural minuets required by the various federal and provincial acts and agencies, there is no better source than *Environmental Assessment in Canada: 1982 Summary of Current Practice* (Couch 1982). For those who wish to know how the divisions are made as to who does what studies for various kinds of projects, it is best to speak with provincial or regional federal administrators. They are also the best sources of information on how it is decided how much effort is to be expended in making an assessment of impact.

Initial Environmental Impact Assessments for Proposed Projects

It is widely agreed that environmental considerations should be part and parcel of the earliest feasibility studies for any proposed large-scale project of potential major impact (e.g. Lash et al. 1974). Some such projects are, of course, virtually harebrained from the start and can be dismissed with only a cursory assessment.

Other projects may be somewhat more plausible and, given favorable economic circumstances, may come to fruition. Schemes for the diversion of major river systems over large geographic distances in North America have often been suggested as a solution for the highly uneven distribution of precipitation over the continent. To date, such diversions have been on a relatively local scale, but it is technically feasible, for example, to divert water from the upper Yukon drainages to the south central United States, and indeed this was proposed in the notorious North American Water and Power Alliance (NAWAPA) scheme. Less melodramatically, the advent of techniques for long-distance transmission of electricity has opened up possibilities for power projects at sites that were once considered too remote to be economical.

For projects such as these, initial environmental impact assessment should be appropriate to the circumstances — broad brush, once over lightly, identifying possible problems and little else. What needs to be done can probably best be accomplished by chatting with people who know the area, consulting the literature and files of government information, and otherwise waiting for the prospects to come into sharper focus. For the proponent of the project, who will eventually carry the burden for a detailed environmental assessment, it may well be sufficient to call an armchair consultant. For government agencies that will eventually be expected to have what is called "baseline" data, any potential major project should occasion some search to see what is known and what initial steps might be taken to prepare for the eventualities of the future.

A large fraction of major project proposals prove to be pie in the sky, and a goodly proportion of the remainder, especially the larger ones, are conceived as much as 20 yr before they are implemented. They can thus serve as an early warning system of the inadequacy of background information. The many visions for northern development in Canada were largely ignored as being just visions, with the consequence that as late as 1975 there was still a dearth of information on renewable natural resources. The early warnings were not heeded (Science Council of Canada 1977) and there has been considerable scrambling in recent years to gain quick understandings.

Unfortunately, this scrambling did not always take advantage of what was known, and led to duplication of past work as well as duplication in the scrambling. This is often the case for large projects. Leaving the literature unexplored and heading for the bush, field parties undertake reconnaissance type surveys with poorly defined objectives. Admittedly, these surveys make those who do them familiar with the ground and the locals, but they are not efficient as scientific enterprises (the standard joke is that the biggest benefactor is Kodak). Any such survey should be preceded by preparation of a provisional statement of likely environmental effects, a statement prepared solely on the basis of materials at hand. Using only library materials, a description of a proposed project, and broad experience, it should be possible for any competent limnologist or fisheries biologist to make some fairly shrewd guesses about likely issues. A reconnaissance survey, while necessary, should flesh out details of local significance and should be of minimal duration. It need not be even of a whole season's duration unless the project is likely to go ahead. With the exception of water flow and climatic records, there is often little to be gained in making observations over a period of years. A thorough prior library study may yield better than 50% of the information that is relevant to an initial environmental impact assessment, and the briefest of reconnaissance surveys may provide as much as 75%.

There are exceptions, of course, to such sweeping generalizations. It may be necessary to obtain detailed information on a particularly valuable resource. For example, a proposed hydroelectric project on the Stikine River, which arises in British Columbia and crosses the Alaska panhandle to the sea, would have potentially profound effects on salmon. The size of these salmon populations, their timing, the location of the spawning and rearing areas, and so on, were little known at the time the proposal was made and are crucial to evaluation of the environmental impact of the project. In this particular case it was not possible to draw upon a sufficient bank of relevant information (though perhaps it should have been) for an adequate preliminary assessment. But even in these exceptional cases a relatively cursory seasonal survey could accomplish a large proportion of what would ensue from a detailed or prolonged assessment. For the exceptional as well as the commonplace there is a robust law of diminishing returns.

The morals to be drawn, as I see it, are twofold: first, that despite early warnings, background studies frequently have been inadequate, and second, that preliminary environmental impact assessments have tended not to be done efficiently. But that is better than having done virtually nothing as background and a minimum by way of preliminary assessment, which was surely the case for many projects that were undertaken in the 1950s. (The construction of dams on the Capilano River, the source of Vancouver's drinking water, and on the Campbell River on Vancouver Island are good examples of the slapdash approach of yesteryear.)

Environmental Assessments of Likely Projects

When a project approaches the day of decision to proceed to implementation, there is frequently a tendency to intensify studies of a kind similar to those that have already been done. Much more detailed information may be collected over the same range of subjects with little gain in understanding, so that the imponderables of the final assessment are often the same as those of the initial assessment. For example, in studies concerned with the possible impact of an oil pipeline in the Mackenzie Valley in the Northwest Territories (Canada), later assessments accumulated increasingly more information about fish distribution, movements, rates of growth, and food habits but provided little evidence that fish would be in any way affected. There was much speculation about the possible effects of oil spills on plankton, benthos, fish, and marine mammals, but it was only after considerable prompting and criticism (e.g. see Schindler 1976) that, belatedly, some relevant experimental studies were conducted. This is but one example from many that might be cited in which more of the same was done instead of pilot projects or direct experiments that might have substituted evidence for speculation.

It is also not uncommon for environmental assessments to have major blind spots. For example, for many years impact assessments of large hydroelectric projects were often seriously

restricted in geographical scope. Most attention was given to obstruction of fish migration at the dam, flooding of spawning areas in the lake(s) and streams of the area to be flooded, and estimation of productivity in the reservoir-to-be. Little attention was given to possible downstream effects such as stream bank erosion and estuarial ecosystems, or to evaluating the effect of the new reservoir on patterns of recreational use. The extensive review by Andrew and Geen (1960) drew attention to many of the potential impacts and did much to encourage more comprehensive assessments.

Concerning downstream effects, the classic Canadian faux pas was the failure to anticipate the effects of a large dam on the Peace River in British Columbia on the waterfowl and muskrat marshes at the confluence of the Peace and Athabaska rivers 800 km downstream from the damsite. The marsh ecosystems at the western end of Lake Athabaska are dependent upon the high spring floods that result when the channel of the Slave River cannot accommodate the combined flow of the Athabaska and the Peace. With the flow of the Peace River controlled by the dam, the annual floods would be much reduced (and were for 3 yr immediately following the completion of the dam).

As a consequence of the failure to foresee these effects, some frantic efforts were made to design devices for creating ice jams and to design systems of weirs, the latter of which to some extent mitigated the effects of the dam on flow regimes (Peace-Athabasca Delta Project Group 1973). There is now a scheme afoot to place a dam in the Slave River. The upstream and downstream effects of this proposal are being extensively examined, so perhaps the same mistake will not be made twice.

The devising of appropriate studies for evaluating long-term effects has been a persistent and nagging problem of environmental assessment. The repercussions of an environmental change on a particular population of organisms are not readily studied beforehand and there is usually little opportunity to conduct experiments on a large enough scale or over a long enough period. Assessments must perforce rely on general principles that emerge from the literature or on experience gained from similar projects. The nature of many ecosystem changes may be fairly reliably predicted from accumulated knowledge and experience gained elsewhere, but predicting the particulars of the relative abundance of various species that are involved in interactions is beyond the present capacities of ecology as a science (for an excellent discussion, see Rigler 1982). The common reaction to this set of circumstances is to allow an environmental evaluator a few pages of rhetoric about long-term effects at the end of the assessment, but otherwise to give such considerations little weight in the final decision.

A modern-day response to this sort of problem is to hope that a variety of mathematical techniques will give insights that are otherwise beyond the reach of our deductive abilities. The mathematical techniques generally fall into the category of matrices that can be manipulated to reveal interactions of various orders, or simulations that can pursue such interactions to their mutual numerical consequences while providing maximum flexibility for considering alternative scenarios. The shortcomings of these techniques are well known; matrix analyses lack a dynamic element, simulations reach conclusions that are only as valid as their assumptions. Both may leave something out. But these are not good reasons for not using them. Especially for the uninitiated, they are immensely valuable for expanding awareness of the properties of complex systems, hence the frequent statement that simulations generate predictions that are "counterintuitive." For the more experienced, they provide a useful way of assessing the relative importance of variables in influencing outcomes (sensitivity analysis) and can thus give guidance to further field work or experiment. The workshop techniques developed by Holling and his colleagues (Holling 1978) have combined modelling with multidisciplinary approaches and have been particularly useful as assessment tools.

In the absence of any convincing technique for assessment of long-term effects, it is frequently suggested that a project be delayed until comprehensive ecological studies can be undertaken. Aside from the fact that such studies have a tendency to go on "forever" (there is always more to learn and perennially there is the "urgent requirement" for just one more year's data), it has to be acknowledged that natural systems being what they are, it is simply not possible to predict their behavior except in fairly general terms. Historical data may be useful, of course, and some routine collection of data to assess natural variability in an ecosystem may enable one to say after the project such things as "there was never anything like it before" or "this was just a natural change." Nevertheless, it is important to appreciate that long-term prior studies will not necessarily give insights into many long-term effects of projects.

A better case for long-term ecological studies can be made in relation to environmental science as a whole, with no constraints with respect to where studies should be conducted, nor what the effect might be of a particular arbitrary perturbation of the environment such as would be occasioned by a particular project. Although many of the same difficulties of time scale apply, there is much to be said for their value and they are strongly advocated as an antidote to the "fire fighting" so characteristic of Canadian environmental science of the last two decades (Vallentyne 1978).

In my view, the key points for study of likely projects are as follows: (1) avoid getting "more of the same" kind of information, (2) stress pilot projects and experiments, (3) consider impacts far from the project and in the long term, (4) do computer simulations, and (5) do not count on long-term, large-scale, beforehand studies to reveal all.

It is to be noted that environmental consulting companies are commonly not ideally equipped to do what is needed. They are often only capable of getting "more of the same" kind of information. They are not generally staffed with the cadre of basic scientists that long-term prediction may require. Pilot projects and experiments, especially on a large scale, usually require an orchestration of effort by the proponent and government agencies. There are few consulting companies that are as yet engaged in ecosystem simulation studies.

There is an obvious reason for this state of affairs: consulting companies can only bid on what they are asked to bid on, which is usually relatively run-of-the-mill, more-of-the-same studies for proponents or public interest groups, or farmed out work for government agencies that are assembling a dossier of relevant material. For a very large megaproject, a large consulting company may orchestrate private and public sector participation for comprehensive assessment, complete with pilot projects and experiments (large-scale oil-related developments come to mind), but that is a managerial role, a consulting horse of a different color.

Assessment of a Project under Construction

There is a saying to the effect that "the dogs bark and the caravan moves on," which always comes to my mind once large-scale projects get under way. It is perhaps understandable

that protesting public groups might give up the battle once lost, moving on to wars against other projects that may still be stopped. It is more difficult to understand why professionals often do the same; perhaps it is because the protestors are demanding studies on the possible effects of those other projects. Whatever the reason, it is lamentably true that there is much better documentation of what was supposed to happen than of what did happen, and even less documentation of what went on during construction.

Another example from the experience with Pacific salmon is particularly apt. All of the species of Pacific salmon die at spawning. It follows that if construction should block migration to the spawning areas for any lengthy period, the population may be wiped out. In the case of pink salmon (*Oncorhynchus gorbuscha*), which almost invariably mature at 2 yr of age, a 2-yr complete blockage may be synonymous with extermination, and because in many river systems there are pink salmon runs only in alternate years ("odd" or "even"), a single year's blockage may suffice. Therefore, it may be critically important to provide for fish passage during construction if the permanent fish passage facilities, however good, are ever to be used. The timing of reservoir filling may also be highly relevant to fish passage both upstream and downstream.

For such projects as oil and gas pipelines and new railways, a large part of the environmental concern may be focussed on the construction activity, which must be closely monitored if avoidable effects are indeed to be avoided. Many possible effects are discussed at length in environmental impact assessments. Effective follow-up to document what effects there were and how they were or were not circumvented is much less in evidence. Part of the difficulty is that the actual workers at the scene of construction are usually not well briefed on what it is they should and should not do; in many instances, neither are the foremen. The best of intentions in the head office can frequently be frustrated at the construction site.

The practical way of getting the job done properly would seem to be to have those with environmental expertise on the site at the time of construction to observe and, if necessary, supervise, but this sort of practice is expensive and demanding of greater reserves of qualified manpower than most environmental agencies can muster, even if someone else is paying for it. In consequence, there are many effects of construction activities that are small and highly local happenings but which in the aggregate constitute an invisible overhead that should be tacked on to the assessment of the environmental impact of almost any major project.

Associated with the need for assessment during construction is the obvious need to do something remedial or mitigating if an unforeseen effect occurs. Curiously, too much emphasis is placed on trying to predict what will happen and too little on what to do if the predictions are not accurate. Such considerations have led to phrases such as "coping with uncertainty" and "crisis management." In layman's terms these phrases mean "play it by ear" and "be prepared." When it comes to particular projects, it means having administrative pathways ready-greased for quick response. Grease, in this context, means anticipated in the budget. All major project budgets should include a few millions of dollars for environmental contingencies during construction.

Assessment of a Project after Construction

If interest in environmental matters does not lag during construction of a major project, it almost certainly will when construction is completed. In British Columbia alone, there are several major reservoirs created by dams built in the last three decades that have yet to be studied limnologically (the chain of lakes flooded by the Nechako Dam, the reservoir created by the Mica Creek Dam on the Columbia River, the Whatshan Lakes reservoir, Williston Lake behind the Bennett Dam on the Peace River). There are literally dozens of lesser reservoirs and hundreds of small reservoirs for which there may have been a prediction of what might happen but virtually no record of what did happen. It may well be that there are reports in government files on many such projects and for one reason or another they have not been published, but certainly there is a small published literature.

Such studies as are published are not usually conceived as comprehensive assessments of impact. Rather, they are concerned with some particular aspect of biology or management. For example, a study may be concerned with physical circulation under various regimes of discharge from a reservoir, or management of reservoir fish populations, or the effectiveness of a particular fish ladder design. Comprehensive, after-the-event assessments of major projects are rare.

Several factors contribute to this deficiency, the chief one that there are many other investigations and research topics that are seen as more important to management or more exciting as science. In Canada at least, some of this lack of interest in "postmortem" studies may stem from the abundance of alternative aquatic resources. If a system of lakes is flooded and its fisheries are largely destroyed, there are still many other systems of lakes to turn to. If a new fishery is created in a reservoir, it is just one more among many. In more arid regions where a reservoir may be much more a focus of recreational activity, such as in the United States midwest and southwest, there has been much more attention to management of artificially created aquatic environments. But even in this heartland of reservoir biology and management there is not much literature that is targeted specifically on the accuracy and adequacy of assessments that were made before a reservoir was created.

There have been some instances in British Columbia where a particular consequence of a project has been closely watched. For example, when the Cleveland Dam was built on the Capilano River, it was decided to truck the coho salmon (*Oncorhynchus kisutch*) to spawning grounds above the reservoir. If this did not work, a hatchery was to be built by the proponents of the dam (Hourston et al. 1955). With the local conservation clubs breathing down their necks, the responsible government officials undertook several investigations. It was discovered that, although the adults were successful in spawning, there was a substantial loss of seagoing smolts at the damsite. A hatchery was built below the damsite (at federal government expense!) and has been a notable success in producing coho.

In a somewhat similar vein, a fairly close watch has been maintained on the effect on salmon of the reduction in flows of the Nechako River as a result of the Aluminum Company of Canada diversion to the Kemano River. But again, this has been the monitoring of a particular effect. A systematic overall assessment has not been undertaken.

The record for follow up is much better if a project involves the discharge of an effluent into a lake or stream. In some ways this is an easier assignment scientifically, for what is upstream remains as a permanent control (of sorts) against which to measure what happens downstream. Moreover, if bad effects

occur, there is some prospect of being able to do something about them. What is surprising, though, is that the consistency with which pollution problems is monitored is not matched by a comparable attention to the aftereffects of water diversions or dams or gravel removals and in general the host of impacts that is not associated with the discharge of effluents.

Small Projects

For every large project that attracts public attention to environmental concerns, there are many smaller projects (another logarithmic series?). To give every application the "full treatment" is evidently impractical. Many of the smaller applications are accordingly approved with conditions imposed only on the strength of assessments by field officers. To monitor each project rigorously is equally impractical, and adverse effects may become known to field officers only through complaints of anglers. The same is true for many other impingements on aquatic environments for which the need for a water licence was not even considered. Any creek in an urban environment is sufficient testimonial to the myriad of small environmental effects that in aggregate can destroy a natural ecosystem.

There is no simple answer to these problems. Public education is useful, of course, and many citizens' groups devote themselves to such causes. Field officers of government departments do more or less systematic patrols. But just as large projects entail an overhead of unanticipated effects, the multitude of small projects entail a burden of degradation on the environment as a whole. The best response is to actively undertake to improve the environment either by rehabilitating disturbed ecosystems or by enhancing natural ecosystems. Why do environmental protection agencies not have a budget for environmental rehabilitation and enhancement?

Assessment and Research

I must conclude from the foregoing that though we have come a long way since the 1950s, there is still some distance to go in the business of environmental impact assessment for projects affecting aquatic resources. There are insufficiencies in the execution of initial studies, there is little direct experimentation to test hypotheses, and there are few rigorous before-and-after (especially after) studies aimed at assessing how good we are at predicting what effects will be. Hilborn and Walters (1981) even go so far as to advocate that "one of the best ways to spend money on baseline studies is to compile a detailed set of retrospective studies." Instead of jumping in a helicopter and looking at the site of a proposed project, they recommend going to some developed sites to see what happened. In this regard, SCOPE Report 2 (Dussart et al. 1972) is an excellent summary of worldwide experience of man-made lakes.

Such a state of affairs suggests that much of the expenditure on environmental impact assessments is not making the contribution it might to scientific knowledge. To some extent it can be argued that the objective of assessment is not to contribute to scientific knowledge so much as it is to foster public knowledge. Certainly the presence of a field party can be a valuable source of technical information as well as a catalyst for local groups who have a desire to make representations about a project in public hearings. Nevertheless, most assessments, if adequately followed through, have substantial potential for adding to the bank of experience that would help make future assessments more sound.

This line of argument leads logically to the conclusion that environmental impact assessment and scientific studies of disturbed ecosystems should probably be conducted as separate but parallel activities. Much that is done to prepare an assessment could be used for scientific purposes but is not because those who collect the information do not see it as their job to make contributions to scientific knowledge.

In a somewhat related vein, Wallace (1981) remarked that there may be conflicts between those administrators who wish to see "publicly-visible research," those to whom the scientists report who may profess a desire for "mission-oriented research," and the scientists themselves who are motivated by their peers to produce "good-quality scientific research" with no constraints on publication. It may be possible to do a piece of good-quality, mission-oriented, publicly visible scientific research, but it is not an easy target when working within the constraints of an assessment of a particular project.

Maintaining the distinction between environmental impact assessment and scientific studies of disturbed ecosystems would perhaps not be necessary if it were possible to manipulate natural ecosystems on the same grand scale as major projects. As it is, of course, the costs of so doing are far too great. It is equally difficult to experiment on a large scale during the course of project construction, to follow a sort of learn-as-you-go procedure in which as far as possible "future options" are preserved. It is often not practical to do only half of a major project nor to delay construction for a long period to evaluate effects up to that point. For better or for worse, most major projects face a relatively early "go" or "no go" decision shortly after construction begins, usually because of the way financing has been arranged. Experimentation is anathema when the banker is waiting for payments.

Wallace (1981) suggested that new institutional homes be found for environmental impact research: within government a Crown Corportion, outside of government an independent research foundation. My view is that there are already many institutions and that changes in attitudes will be more important. For example, a large part of the present inadequacy can be traced to the different meanings attached to the word "research." For some, research is virtually synonymous with personally discovering something, no matter how many people already know it. For others, research means only the process by which some new knowledge is gained that is new to everybody. What is needed is more research of the latter kind if we are to improve our understanding and predictive capabilities for aquatic ecosystems.

Environmental impact assessment as presently undertaken is unlikely to have much impact in resolving our ignorance, and for that reason is not likely to lead to any substantial program or policy changes by governments. By contrast, research that will lead to new knowledge may have a profound effect on programs and policies. Virtually all of the environmentally oriented legislation of the past century in all parts of the world has roots in the advance of scientific understanding in general and the growth of ecological science in particular. With respect to freshwater ecosystems, Canada has been a major contributor, as is appropriate considering our national wealth of aquatic resources. It will be important to future use of these resources to encourage major research enterprises on natural and disturbed

ecosystems independently of the need to undertake the routines of environmental impact assessment for major projects.

Postscript

After the foregoing was written, the comprehensive review of Beanlands and Duinker (1983) was published. This extensive work, based on wide discussions, is in many respects an admirable critique of the environmental impact assessment process in Canada. The document is addressed to the full spectrum of possible environmental impacts and the language tends therefore to a high level of generality and abstraction. Nevertheless, there are many points that have strong bearing on large projects affecting lakes and streams, and many of the points that I have raised are treated comprehensively. For example, there is strong emphasis on the need for monitoring after a project is completed.

More important, from the perspective of this essay, in the more than 100 pages of the report there is no mention of how impact assessments might become contributions to knowledge, and no consideration of the need either for research on impact assessment or long-term research on aquatic ecosystems. These topics were said not to be within the scope of the project. It is to be hoped that before too long these topics can be given the consideration they deserve, especially in the budgeting processes of government.

Acknowledgments

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BOOK REVIEWS/COMPTES RENDUS

Guide to the Early Stages of Marine Fishes Occurring in the Western North Atlantic Ocean, Cape Hatteras to the Southern Scotian Shelf. *Michael P. Fahay. J. Northw. Atl. Fish. Sci. 4: 423 p. Northwest Atlantic Fisheries Organization, Dartmouth, N.S. 1983. $20.00 (softcover), $30.00 (hardcover).*

This guide to eggs and larvae of fishes from the continental shelf and oceanic waters of the western North Atlantic is a welcome addition to the working literature on ichthyoplankton identification. It is the best comprehensive treatment of the topic for the designated region and it also is a valuable aid to identification of fish larvae throughout the Atlantic Ocean. For some oceanic families the guide can be used effectively for ichthyoplankton collected anywhere in the world. The guide has descriptions and illustrations of eggs and larvae of 290 species in 92 families. In a few cases, estuarine species are included to allow comparison with similar shelf or oceanic species.

As in most guides, descriptions and illustrations are based on previously published work. The quality of illustrations is generally excellent, sometimes because the author redrew original illustrations to improve them or make them conform to the guide's format. Left-hand pages give descriptive material, including notes on spawning, eggs, and larvae. Meristic data are tabulated and easily located on the page. When appropriate, tables of comparative data on similar species are provided. The larval illustrations often have notes appended to point out critical identifying characters. A feature that will be appreciated by users of the guide is the attempt by the author to correct errors that sometimes were made in the original descriptive papers. The corrections will help to stop perpetuation of these errors in future ichthyoplankton identification.

Introductory pages are included for families or (sub)orders with numerous species (e.g. Gonostomatidae, Myctophidae, Scombridae, Anguilliformes, Gadiformes) in which useful tables of meristic and morphometric information are provided to help distinguish taxa. Pigmentation, spination, and sizes at which fins develop are used throughout the guide to aid identification. Photophore patterns and sequence of development are given for Gonostomatidae and Myctophidae. A section on "miscellaneous" other larvae provides information and illustrations on taxa not commonly found in the region of concern. Other useful features of the guide are its taxonomic index, glossary, summary tabulations of developmental characters at the ordinal level, and summary tables of meristic characters for all taxa in the guide. References through 1982 will give users access to primary literature. The author sought advice of many experts on ichthyoplankton during preparation of the guide which has contributed to its high quality.

I have used the guide in my research on ichthyoplankton in the Florida Current and found it very useful even though the Straits of Florida is outside the Hatteras to Scotian Shelf area. I was impressed by the high quality of the printing and the attractiveness of the book. It is remarkably free of typographical or printing errors, further tribute to the author and to the Northwest Atlantic Fishery Organization. In my opinion the guide is a required addition to the laboratory workbenches of ichthyoplankton identifiers. I predict that it will become a standard reference, not only for the western Atlantic, but worldwide because of its comprehensive coverage of oceanic species. The author and the publisher might wish to consider occasional updates to the guide which could consist of supplements published perhaps on a 5-yr basis or when significant new material is available in the primary literature.

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(R528)

ANNOUNCEMENTS/AVIS

ICSEB - III
Third International Congress of Systematic and Evolutionary Biology, 1985

The Third International Congress of Systematic and Evolutionary Biology will be held on 4–10 July 1985 at the University of Sussex, near Brighton, England, and is to be sponsored by the Royal Society, British Ecological Society, Linnean Society, Palaeontological Association, and Systematics Association.

The major aims of this Congress are to encourage and facilitate the integration of the work of biologists in adjacent or overlapping fields in the general area of systematic and evolutionary biology.

The following Congress symposia are being organized: Symbiosis in Evolution; Conservation of Tropical Ecosystems; Biogeographic Evolution of the Malay Archipelago; Adaptation Aspects of Physiological Processes; Co-evolution in Ecosystems and the Red Queen Hypothesis; Angiosperm Origins and the Biological Consequences; The Measurement of Rates of Evolution; Molecular Biology and Evolutionary Theory; Co-evolution and Systematics; Molecules vs. Morphology in Phylogeny: Conflict or Compromise?; Random and Directed Events in Evolution; Biochemical Innovation in Microbial Communities.

There will also be Special Interest symposia on other topics, as well as contributed papers, films, and poster papers.

Anyone wishing to be placed on the mailing list, or to suggest topics for Special Interest symposia, or to contribute to any of the Congress symposia listed above, should write to:

Professor Barry Cox
c/o ICSEB Congress Office
130 Queen's Road
Brighton, Sussex BN1 3WE

CISEB - III
Troisième Congrès International de Systématique et d'Évolution Biologique, 1985

Le troisième congrès international de systématique et d'évolution biologique se tiendra du 4 au 10 juillet 1985 à l'université du Sussex, près de Brighton (Angleterre). Le congrès se tiendra sous l'égide de la *Royal Society*, de la *British Ecological Society*, de la *Linnean Society*, de la *Palaeontological Association* et de la *Systematics Association*.

Le congrès vise surtout à favoriser et à faciliter l'intégration des travaux de biologistes œuvrant dans des domaines connexes ou chevauchants de la systématique et de l'évolution.

On prépare actuellement les colloques suivantes : Symbiose et évolution; Conservation des écosystèmes tropicaux; Evolution biogéographique de l'archipelmalais; Aspects adaptatifs des processus physiologiques; Coévolution au sein d'écosystèmes et l'hypothèse de la «reine rouge»; Origines des angiospermes; les théories de l'évolution; Coévolution et systématique; Molécules et morphologie en phylogenèse : conflit ou compromis?; Evénements aléatoires et déterministes en évolution; Innovation biochimique chez les communautés bactériennes.

On tiendra aussi des colloques spécialisés sur d'autres sujets et des réunions pour la présentation de communications écrites, de films et d'affiches.

Prière de communiquer à l'adresse ci-dessous pour être inscrit sur la liste de distribution, ou pour présenter des sujets pour les colloques spécialisés ou une communication à l'un des colloques mentionnés plus haut :

Professor Barry Cox
c/o ICSEB Congress Office
130 Queen's Road
Brighton, Sussex BN1 3WE

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

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Effect of discharge rate on biotic and abiotic chemical flux in an acidified stream **R. J. Hall and G. E. Likens**

Age at first maturity and early expression of oocyte recruitment processes in two forms of Atlantic salmon (*Salmo salar*) and their hybrids **A. M. Sutterlin and D. MacLean**

Multivariate morphometric variability in Pacific salmon: technical demonstration **G. A. Winans**

Responses of Pacific herring, *Clupea harengus pallasi*, to some underwater sounds **A. L. Schwarz and G. L. Greer**

Insights from the markets for limited entry permits in Alaska **J. M. Karpoff**

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Meteorological and hydrographic regulation of year-class strength in capelin (*Mallotus villosus*) **W. C. Leggett, K. T. Frank, and J. E. Carscadden**

Three-dimensional prey reaction field of the juvenile coho salmon (*Oncorhynchus kisutch*) **R. L. Dunbrack and L. M. Dill**

Environmental controls of marine food webs: food habits of seabirds in the eastern Chukchi Sea **A. M. Springer, D. G. Roseneau, E. C. Murphy, and M. I. Springer**

Effects of fluctuations in hydrographic conditions on year-class strength of American shad (*Alosa sapidissima*) in the Connecticut River **V. A. Crecco and T. F. Savoy**

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Sites of uptake of geosmin, a cause of earthy-flavor, in rainbow trout (*Salmo gairdneri*) **J. From and V. Hørlyck**

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Selective predation for vertebral number of young sticklebacks, *Gasterosteus aculeatus* **D. P. Swain and C. C. Lindsey**

Size selectivity of passive fishing gear: a correction for encounter probability applied to gill nets **L. G. Rudstam, J. J. Magnuson, and W. M. Tonn**

Method of solving for natural mortality rate for stock components with different schedules of growth and mortality **J. F. Caddy**

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On group testing for diseases **J. M. Hoenig and W. D. Lawing**

Reply **D. D. Worlund**

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The biology of Crustacea — environmental adaptations (Vernberg and Vernberg) **G. Jamieson**

Chironomidae of the Holarctic region — Part 1. Larvae (edited by T. Wiederholm)

A review of the genera *Doithrix* n. gen., *Georthocladius* Strenzke, *Parachaetocladius* Walker and *Pseudothocladius* Geotghebuer (Diptera: Chironomidae, Orthocladiinae) (Supplement No. 20) (O. A. Saether and J. E. Sublette)

A catalogue of chironomid genera and subgenera of the world including synonyms (Diptera: Chironomidae) (Supplement 17) (P. Ashe) **R. D. Kathman**

Control processes in fish physiology (edited by J. C. Rankin, T. J. Pitcher, and R. Duggan) **J. G. Eales**

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Journal canadien des sciences halieutiques et aquatiques

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